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EFFECTS OF ADDED SUGARS AND NITROGEN ON NODULATION OF LEGUMES

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INTRODUCTION

An optimum light intensity is necessary for maximum nodulation in legumes [12 13 28 32 33]. The depressing effect of too low a light intensity may be partially offset by growing the legume in an atmosphere enriched with CO_2 from 0.03 per cent (air) to 0.1 per cent [52], or by addition of sucrose, glucose or maltose to the growth medium (Georgi *et al.* [15] and others: refs. quoted by Allison [2] and Wilson [53]). The depressing effect of too high a light intensity may be counteracted, either by giving combined nitrogen, or by a short period of shading [53]. Excessive carbohydrate formation induced by high light intensity, or by high CO_2 or sugar concentrations, may lead to decreased fixation of nitrogen. If nodulated plants are placed in the dark, nodule formation ceases and nodules already formed, degenerate [38 51]. *Leucena glauca*, red clover and soybeans do not form nodules in the dark unless provided with sugar [37]. The red pigment of pea nodules changes to green when the plant is subjected to complete darkness for 2 to 3 days [43]; the decomposition of the haemoglobin in soybean nodules, on the other hand, is very slow and takes some weeks [46].

Although nodulation in the dark is often reported to be stimulated by addition of sugar to the medium, it did not take place with Canada field peas (*Pisum arvense*) in the experiments of McGonagle [24].

According to Diener [7], a decrease in the number of nodules in peas does not take place until the light is reduced to less than 50 per cent of the normal light intensity. However, the size of the nodules is reduced by quite a moderate decrease in the light intensity.

Varieties of plants adapted to long and short days develop most effectively when grown in their appropriate length of day [20 35]. The reproductive type of growth induced in peas by a 15 to 17-hour day and in soybean by an 8 to 12-hour day decreased nodule formation (by weight), but increased nitrogen fixation per gram of nodule as compared with the vegetative type of growth induced in peas by an 8-hour day and in soybean by a 15 to 17-hour day [9].

Bonnier and Sironval [4] found that soybeans formed large pink nodules in a 16-hour day, but in an 8-hour day the nodules were small or did not develop at all.

Numerous papers deal with the depressing effect of combined nitrogen on production, size and function of nodules, which varies not only with the nature of the combined nitrogen [10 34 50] and the growth substrate [11 18], but also with the species of host [10 27]. In the field, large amounts of combined nitrogen are required to produce complete suppression of nodules [30].

The depressing effect of abundant nitrogen can largely be cvercome by promoting photosynthesis with additional light or additional CO_2 [12 16 47 53], or by adding carbohydrates such as sucrose, dextrose or laevulose to the growth substrate (Wilson [51], Allison and Ludwig [1], Ludwich and Allison [21] and others). For these reasons it is very probable that too low a carbohydrate nitrogen ratio in the plant, leading to inadequate carbohydrate supply in the roots, is responsible for decreased nodulation in the presence of abundant combined nitrogen [1 2 19 53].

It should be emphasized, however, that the results of experiments showing a favourable effect of carbohydrates on nodulation, when added to the growth substrate in the presence of large amounts of nitrogen, are only convincing when the plants are grown under aseptic conditions. It is very likely, for instance, that in the experiments of Allison and Ludwig [1], the medium was invaded very soon by micro-organisms from the air which, in the presence of sugar, may have developed very rapidly and consumed a large part of the combined nitrogen added to the sand.

According to Weir [48], in aseptic culture, the effect of carbohydrates appears to be entirely plant physiological, whereas in non-sterile soil culture, this is over-shadowed by effects of the C/N balance, evoked in the first instance by saprophytic soil micro-organisms. Under aseptic conditions, nodulation in *Medicago sativa* L. was decreased by the addition of a range of carbohydrates at a concentration of 1 per cent. This decrease was more marked with mono- and di-saccharides than with tri- and poly-saccharides *.

That the amount of nitrogen present in the plant tissues may exert an internal effect on nodulation is evident from the work of Chailakhian and Megrabian [6], who, employing a technique in which the upper part of the roots of legumes developed in sand and the lower part in culture solution, observed that nodule formation in the sand was considerably inhibited when nitrogen was added to the solutions. MacConnell and Bond [23], working with plants which were allowed to take up combined nitrogen for a certain period before inoculation was carried out, arrived at the same conclusion.

Some investigators, however, have obtained experimental results suggesting that nodulation may also be affected by nitrogen or carbohydrates locally in the growth substrate apart from changing the C/N ratio in the plant.

Root-hairs are not infected unless deformed by a secretion of the nodule bacteria. Thornton [39] showed that root-hair deformation in the presence of sterile bacterial secretions was checked by nitrate, while the inhibiting

* For a recent paper on this subject see *Appendix* (p. 112).

effect of nitrate upon the action of the bacterial secretions was completely neutralized by dextrose.

By using the divided-root technique it has been shown that nitrate supplied to one part of the roots had no effect on the formation of nodules on the other part [8 14 44 45 50]. According to Wilson [50], this proves that the effect of nitrate is local in character.

Raggio *et al.* [31], who developed a special technique for growing excised roots of legumes *in vitro*, found that nitrate added to the inorganic growth medium significantly reduced nodule formation, while nitrate supplied via the base of the root exerted no such inhibitory effect.

In contrast to the results mentioned above, small quantities of combined nitrogen have sometimes been found to favour infection [7 20 22 33] and nitrogen fixation [5 12 29].

Norman [24] and Norman and Krampitz [26] observed that soybeans did not make maximum growth when dependent solely on the fixation mechanism for their nitrogen supply. Thornton [40 41 42], by the use of tracer technique, noted that red clover, sweet clover and soybeans can effectively use more nitrogen than is provided by fixation. Lyons and Early [22] showed that whether or not combined nitrogen exercises a favourable effect on the yield of soybeans depends on climatic conditions. A favourable effect of fertilizer nitrogen was also found by Allos and Bartholomew [3] in various legumes excluding soybeans. By the use of labelled nitrogen it was shown that the mean recovery of fertilizer nitrogen was 55 per cent in legumes and 85 per cent in grasses. Fixation of atmospheric nitrogen decreases with increasing rates of fertilizer nitrogen. Of course a favourable effect of combined nitrogen on the growth of legumes may be expected when the fixation of nitrogen is performed by moderately effective strains of Rhizobium.

The purpose of the present investigation was to provide more convincing evidence on the controversial results mentioned above, from which it might be possible to decide whether the C/N ratio in the plant or in the growth substrate must be held responsible for the influence on nodulation and N-fixation.

The effect of sugars and of combined nitrogen on nodulation and nitrogen fixation has always been tested by adding these substances to the growth medium. In the first experiment described in this paper also, the effect of various concentrations of glucose and of sucrose added to the growth substrate was studied on nodulation of white clover and lucerne, grown in agar culture during the winter months, in the presence or absence of additional light. In a second experiment, however, the effect of 0.5% sucrose solution, sprayed at intervals on the leaves of peas, was studied also in winter time, in plants grown aseptically in quartz sand, in the presence or absence of additional light. In a third trial, the influence of urea

solutions, sprayed at intervals on the leaves, was investigated in pea plants grown aseptically in quartz sand during February and March 1958, in the presence of additional light.

The nitrogen content of the pea plants was determined according to Kjeldahl-Gunning.

EXPERIMENT 1

Experimental method

Clover plants (*Trifolium repens*) and lucerne plants (*Medicago sativa*) were grown in test tubes plugged with cotton wool, on agar of the following standard composition:

K_2HPO_4	500 mg	Soil extract	500 ml
$MgSO_4.7H_2O$	500 mg	Tap water	500 ml
$CaCO_3$	750 mg	Agar	7.5 g

The soil extract was made by boiling 1 kg clay soil for half an hour in 2 l water. After filtration this extract was made up to two litres.

For the clover plants, the standard medium was provided with 0, 0.5, 1, 2 or 5% glucose, or with 0, 0.5, 1, 2 or 5% sucrose. For the lucerne plants, concentrations of 0, 0.2, 0.5, 1 and 2% glucose or sucrose were tested, since in a preliminary experiment started on 5th October, it had been shown that nodulation in lucerne plants was checked entirely by a concentration of 5% glucose or sucrose.

After addition of sugar, the standard medium was boiled and filtered to obtain a clear substrate. The media were sterilized for half an hour at 120° C. Each test tube (diam. 1.5 cm) was provided with 10 ml medium and on 7th November, three inoculated seeds, which had been previously sterilized, were sown in each tube. The seeds were sterilized by shaking for three minutes in a soap solution, rinsing twice with tap water, then shaking for four minutes in 96% alcohol and rinsing for two minutes in tap water. Finally they were treated for four minutes in a 1% bromine solution and then washed with sterile water.

For each plant species and for each sugar concentration 24 cultures were used, and for the controls 60 cultures. The culture tubes were placed in the greenhouse in cylindrical holes bored in wooden blocks to a depth sufficient to cover the medium. Within each treatment, half the cultures were given additional light by placing them on a 3.44 × 0.98 m bench 60 cm below four 100-watt Philips high-pressure mercury lamps supplemented by three 20-watt TL lamps. In addition to normal daylight, the plants on this bench were exposed to the artificial light for 15 hours each day, from 6 a.m. to 9 p.m.

Results

Figure 1 shows the effect of various glucose concentrations on nodulation of clover plants, (A) in the absence of and (B) in the

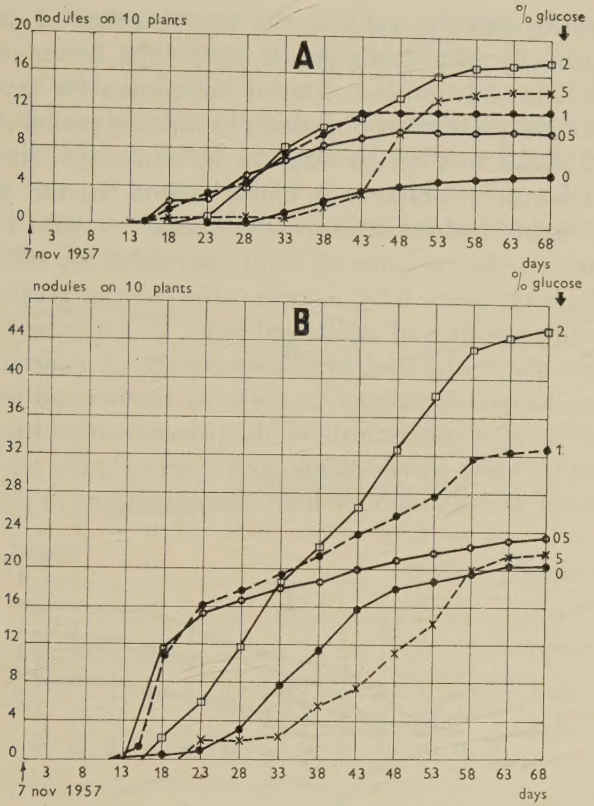

Fig. 1. Influence of various concentrations of glucose on nodule formation in clover grown in winter, on agar, (A) in the absence and (B) in the presence of additional light.

presence of additional light. In the absence of glucose, nodulation started about 10 to 15 days later in plants grown without additional light as compared with those grown with additional light. In the absence of glucose and without additional light, several plants did not form nodules at all, and the total number of nodules was much smaller than in the presence of additional light. Both in the absence as well as in the presence of additional light, nodulation was stimulated by glucose. In the first period of plant growth especially, the concentrations of 0.5 and 1% glucose stimulated nodule formation. Ultimately, however, the highest number of nodules was obtained in the presence of 2% glucose. Although, in the absence of artificial light, nodule formation started earlier in certain plants which had

been provided with 5% glucose, the total number of nodules increased markedly only after a period of 43 days. In the presence of additional light, 5% glucose delayed nodulation for several days and checked the production of nodules in the first period of 58 days.

Figure 2 shows, in a similar way, the influence of various sucrose concentrations on nodulation in clover plants. In the absence of additional light, nodulation was stimulated by 0.5, 1 and 2% sucrose and, in the presence of additional light, by 0.5 and 1 % sucrose. In both cases, 0.5% sucrose effected the greatest stimulation. In the presence of additional light, 2% sucrose stimulated nodulation only in the first period of growth. A concentration of 5% sucrose delayed nodulation in both treatments and reduced the numbers of nodules consistently in the presence of additional light. Nodulation in white clover plants was checked more by high concentrations of sucrose than by high concentrations of glucose.

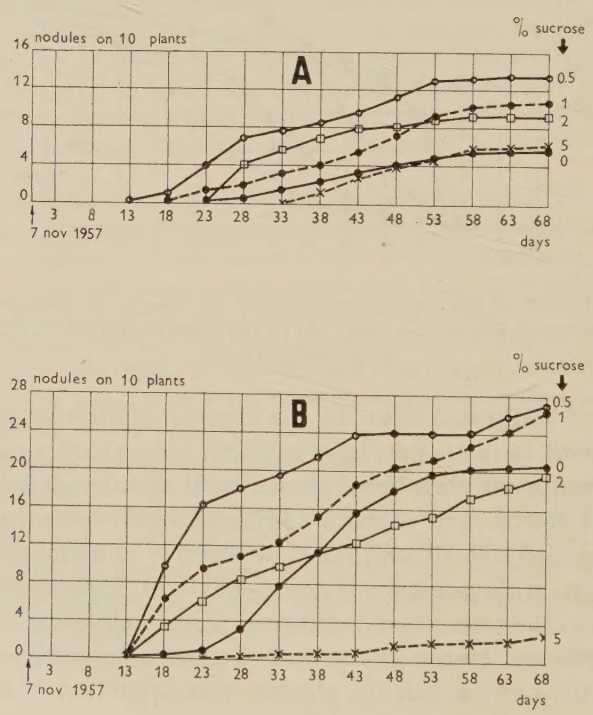

Fig. 2. Influence of various concentrations of sucrose on nodule formation in clover grown in winter on agar, (A) in the absence and (B) in the presence of additional light.

In Figure 3 the results are shown of the effect of various glucose concentrations on nodulation in lucerne plants. In contrast with the clover plants, nodulation was not delayed in the absence of additional light, although ultimately, the total number of nodules was markedly higher in the presence of additional light. Moreover nodulation was not stimulated by glucose in the growth substrate. On the contrary, nodulation was checked by all the concentrations used, even by 0.2% glucose.

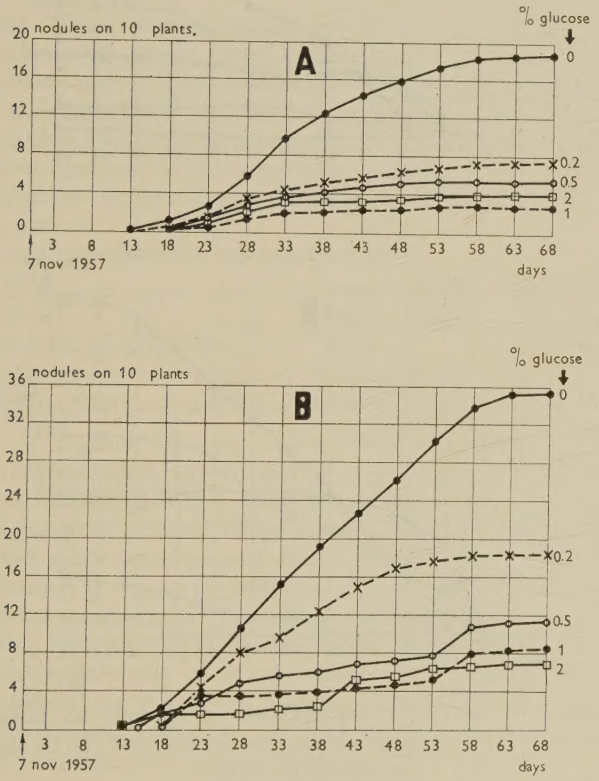

Fig. 3. Influence of various concentrations of glucose on nodule formation in lucerne grown in winter on agar, (A) in the absence and (B) in the presence of additional light.

Finally, Figure 4 shows the results obtained with sucrose on nodulation in lucerne plants. In both treatments a stimulation of nodule formation was only observed in the early period of plant growth, in the presence of 0.2 and 0.5% sucrose. As was the case

with glucose, a decrease in total number of nodules was ultimately obtained with all concentrations of sucrose, both in the absence as well as in the presence of additional light. In both treatments nodulation in lucerne was delayed for several weeks in the presence of 2% sucrose.

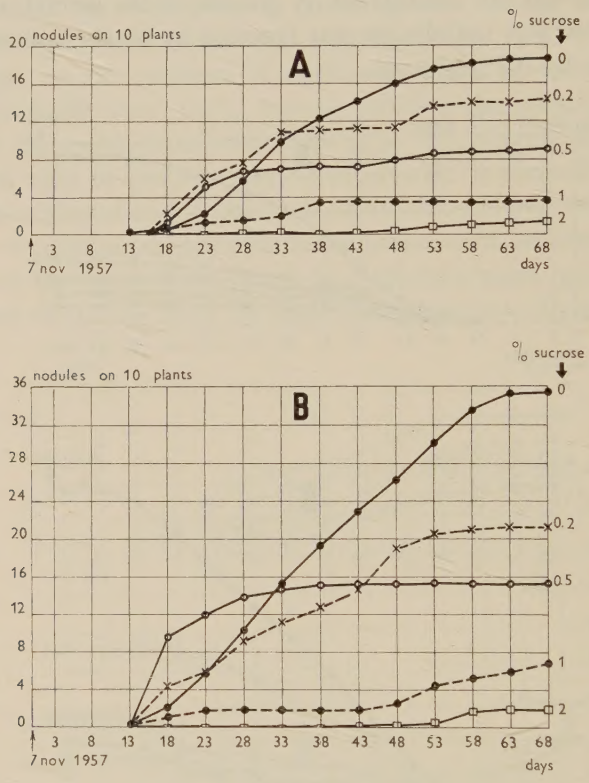

Fig. 4. Influence of various concentrations of sucrose on nodulation in lucerne grown in winter on agar, (A) in the absence and (B) in the presence of additional light.

When nodulation was reduced, or nodules were absent, plants showed poor growth and signs of nitrogen deficiency. Glucose and sucrose at concentrations of 1 and 2% gave rise to thick fleshy roots, especially in lucerne. Addition of 0.5, 1 and 2% glucose or sucrose led to the formation of a red pigment in the stems of lucerne. This effect was more pronounced in the presence of additional light. A red pigmentation was also found at a concentration of 0.2%

sucrose but only in the presence of additional light, while 0.2% glucose did not produce such a pigmentation, neither in the presence nor in the absence of additional light. The presence of a red pigment in the stems indicates a high concentration of carbohydrates in the plant tissue.

EXPERIMENT 2

Experimental method

Pea plants (*Pisum sativum* var. Pauli) were grown aseptically in Weck glass jars containing 2200 g moist quartz sand covered with about 1 cm sterile paraffined quartz sand, as described by Wieringa and Bakhuis [44]. A layer of this paraffined sand acts like a cotton plug, retaining all contaminating germs. This is a simplification of the method used by Gerretsen [1]. The seeds were sterilized in the same way as described in Experiment 1 and then pregerminated on agar plates. Only germinated seeds that proved to be sterile were used.

Each jar was provided with the following salts:

K_2SO_4	1 g	Ferric citrate	200 mg
$CaCO_3$	1 g	$MnSO_4.4H_2O$	2 mg
$Ca(H_2PO_4)_2.3H_2O$	0.6 g	H_3BO_3	0.5 mg
NaCl	0.3 g	$CuSO_4.5H_2O$	0.1 mg
$MgSO_4.7H_2O$	0.4 g	$ZnSO_4.5H_2O$	0.1 mg
		$Na_2MoO_4.2H_2O$	0.05 mg

Of the 48 cultures used, 24 were provided with 100 mg NH_4NO_3.

The jars were covered with a sheet of cellophane paper and sterilized at 120° C for a period of 2 hours. When cooled down to room temperature, the pregerminated seeds were planted (after having been inoculated with a diluted suspension of two active *Rhizobium* strains) and immediately covered with paraffined quartz sand. Paraffined sand was made as follows: 10 g paraffin with a high melting-point were dissolved in 1 l benzol. This solution was mixed thoroughly with 10 kg sand. After evaporation of the benzol, the sand was sterilized for 2 hours in a dry sterilizer at 160° C in Erlenmeyer flasks closed with cotton wool.

In each jar 3 plants were grown from 1st October until 15th November 1957. The moisture content was maintained at 65% of the water-holding capacity by adding sterile tap water through a glass tube inserted in the jar and plugged with non-absorbent cotton wool. This water was siphoned over from a glass container through a rubber tube and a hollow needle attached to the end by inserting the latter between the cotton wool plug and the wall of the tube (Figure 5). The needle was previously flamed for a moment, and while still hot, inserted between the cotton wool plug and the side of the tube so as to kill any micro-organisms which might otherwise be introduced by contact with the outer side of the cotton wool. In this way it was not necessary to remove the cotton wool whereby an important source of con-

tamination was eliminated. The flow of water from the container was regulated by means of a clip fixed on the rubber tube. The rubber tube and needle were previously sterilized together with the container. During sterilization, the needle was enclosed in a test tube, the mouth of which was closed with cotton wool.

In this experiment the effect was studied of five successive sprayings of the leaves with 0.5% sucrose solution at intervals of 3 to 4 days. The first spraying was performed on 29th October, the last on 12th November. Half the number of plants was treated in this way. Spraying was carried out at the rate of 100 ml/m² soil surface by means of a paint sprayer, and the surface of the sand was covered with non-absorbent cotton wool to protect it from

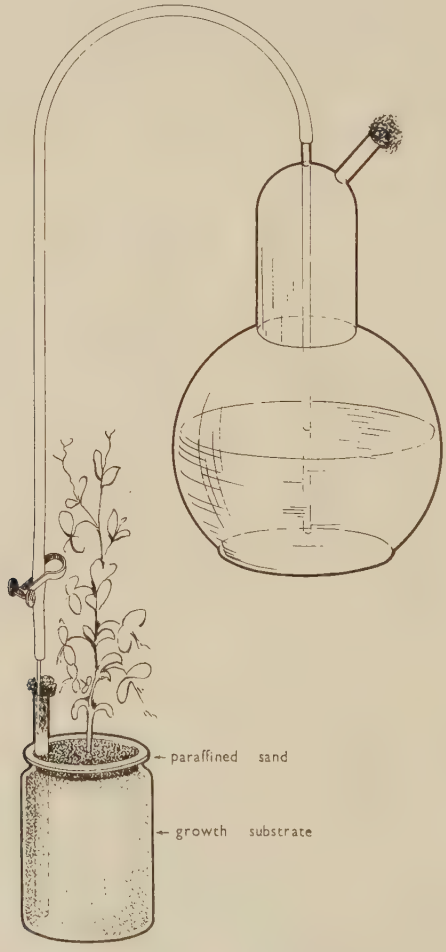

Fig. 5. Provision of sterile water to plants grown aseptically in sand.

contamination with the sugar solution. Half the number of jars within each treatment received additional artificial light corresponding to a 15-hour day as described in Experiment 1.

Results

The results obtained with the plants grown without combined nitrogen are presented in Table 1.

TABLE 1

Influence of 5 successive sprayings of pea plants with 0.5% sucrose solution (plants grown from 1st Oct. to 15th Nov. 1957 in the absence of combined nitrogen. First spraying 29th Oct., last 13th Nov.; average of 18 plants per treatment)

Characteristic measured	Without additional light		With additional light	
	Not sprayed	Sprayed	Not sprayed	Sprayed
Plant dry weight (mg)	250 ± 17	269 ± 8	388 ± 18	365 ± 19
Number of nodules	18	17	47	32
Dry weight of nodules (mg). .	10.9 ± 2.9 (0.60) *	26.5 ± 4.8 (1.56) *	65.5 ± 10.4 (1.39) *	62.0 ± 15.5 (1.94) *
Mean value for nodule weight as a percentage of whole plant weight	4.4	9.9	16.9	16.9
N green parts (% D.Wt.). . .	3.40	4.38	3.69	3.47
N roots (% D.Wt.)	1.98	2.14	2.25	2.21
N per plant (mg) **	7.16	9.83	13.42	12.16
N fixed per g of nodule (mg)***	36	115	102	87

* Mean weight per nodule in brackets.
** Nodules included.
*** After subtraction of nitrogen present in the seed.

The additional light has increased the weight of plants and nodules and also the number of nodules. With additional light, the mean value for nodule weight (as a percentage of the whole plant weight), the nitrogen content, and the total amount of fixed nitrogen were greater than in the plants grown without additional light and which had not been sprayed with a sugar solution. When the sprayed and unsprayed plants grown without additional light are compared, it is seen, that although the plant weight and the numbers of nodules were practically the same in both treatments, the weight of nodules, the mean weight per nodule, the mean value for nodule weight as a percentage of the whole plant weight, and the amount of nitrogen fixation were markedly increased by spraying with sugar solution. In the cultures grown with additional light,

the numbers and total weight of nodules and the fixation of nitrogen were less for the sprayed plants, though the mean weight of the nodules was greater.

By subtracting the nitrogen present in the seed (about 6.77 mg) from the nitrogen present in the plant, the nitrogen fixed by each plant and the nitrogen fixed per gram of nodules could be calculated. From the data shown in the table, it may be seen that the smallest amount of nitrogen fixed per gram of nodules was found in the unsprayed plants grown without additional light. In the sprayed plants grown without additional light, the amount of nitrogen fixed per gram of nodules has increased to the same level as in the unsprayed plants grown with additional light. In the plants grown with additional light, spraying with sugar solution has obviously depressed the amount of nitrogen fixed per gram of nodules.

In Table 2, the results are given for the plants supplied with combined nitrogen.

TABLE 2

Influence of 5 successive sprayings of pea plants with 0.5% sucrose solution (plants grown from 1st Oct. to 15th Nov. 1957 in the presence of combined nitrogen and sprayed between 19th Oct. and 13th Nov.)

Characteristic measured	Without additional light		With additional light	
	Not sprayed	Sprayed	Not sprayed	Sprayed
Plant dry weight (mg)	233 ± 13	277 ± 18	518 ± 29	347 ± 36
Number of nodules	13	15	39	18
Dry weight of nodules (mg). .	11.9 ± 3.5 (0.91) *	22.1 ± 5.4 (1.47) *	67.6 ± 8.0 (1.73 *)	30.2 ± 8.1 (1.68) *
Mean value for nodule weight as percentage of whole plant weight	5.1	8.1	13.1	8.7
N green parts (% D.Wt.). . .	5.62	5.10	4.35	4.95
N roots (% D.Wt.)	2.97	2.92	2.61	3.15
N per plant ** (mg)	11.35	12.53	21.02	15.74

* Mean weight per nodule in brackets.

** Nodules included.

As in the treatment without combined nitrogen, additional light has increased the yield of the plants, the number and weight of the nodules and the mean value for nodule weight expressed as a percentage of the whole plant weight. In the presence of additional light, the addition of combined nitrogen has increased the weight of those plants not sprayed with sugar solution. Added nitrogen

has also increased the percentage nitrogen content of the green parts and roots (especially in the absence of additional light), besides the total nitrogen content. Although the added nitrogen has decreased the number of nodules, the weight of the nodules on the plants which have not been sprayed with sugar solution has not been reduced, resulting in a greater mean weight per nodule. On the other hand, nitrogen added to the plants sprayed with sugar solution has decreased both the number and dry weight of nodules, resulting in a decreased mean weight per nodule. As in the case of the plants grown without added combined nitrogen, spraying with sugar solution has increased the dry weight of the nodules, the mean weight per nodule and the mean value for nodule weight expressed as a percentage of whole plant weight in the plants grown without additional light. However, the increase was relatively greater in the absence than in the presence of added nitrogen.

EXPERIMENT 3

Experimental method

Pea plants (*Pisum sativum* var. Pauli) were grown aseptically in the greenhouse, in the same way as described in the former experiment, in Weck glass jars containing 2200 g moist quartz sand. The composition of the medium was the same as in Experiment 2 without addition of combined nitrogen. In each jar, 3 inoculated plants were grown in the presence of additional light from 4th February until 19th March 1958 when the first blossoms appeared.

The plants were sprayed with various concentrations of urea solution on 21st and 25th February and on 1st, 5th and 10th March. The solutions were supplemented with 0.1% Shell wetting agent and atomized by means of a paint sprayer at the rate of 100 ml/m^2 soil surface. Contamination of the sand was prevented by covering the sand with non-absorbent cotton wool.

The following treatments were carried out:

1. Untreated (20 jars).
2. Leaves sprayed with 0.5% urea solution (16 jars).
3. ,, ,, ,, 1% ,, ,, (16 jars).
4. ,, ,, ,, 2% ,, ,, (16 jars).
5. ,, ,, ,, 4% ,, ,, (16 jars).
6. ,, ,, ,, 8% ,, ,, (16 jars).

After harvesting, the plants were rinsed in water to remove possible remnants of urea from the surface of the leaves. Nodules, roots, and tops were weighed separately after drying at 105° C.

Results

Obviously the concentration of 8% urea solution was too high as this caused a rather severe scorching of the leaves. The final sprayings with 4% urea solution caused only a slight scorching at the borders of certain individual leaves in a few plants.

Table 3 shows the influence of the various treatments on yield, number of nodules, content of nitrogen, and uptake of nitrogen. The total dry weight of the plants was not increased by the sprayings and probably decreased with the 8% concentration of urea. The total weight of nodules decreased gradually with the use of increasing concentrations of urea. The number of nodules was decreased markedly by the sprayings; even the lowest concentration of 0.5% urea solution exerted this suppressing effect. At this low concentration the mean value for nodule weight expressed as a percentage of whole plant weight, was the same as in the untreated plants; at higher concentrations this value decreased gradually.

TABLE 3

Effect of 5 successive sprayings of pea plants with various concentrations of urea solution (plants gro from 4th Feb. until 19th March 1958 without combined nitrogen in the medium)

Characteristic measured	Urea concentration (%)					
	0	0.5	1.0	2.0	4.0	8.
Top dry weight (mg). . .	337.4 ±14	302.3 ±15	308.1 ±14	334.1 ±16	343.7 ±16	319.2
Root dry weight (mg) . .	84.6 ±3	71.8 ±2	72.1 ±3	81.9 ±3	74.2 ±3	60.7
Top-root ratio	3.99	4.21	4.27	4.07	4.63	5.26
Nodule dry weight (mg) .	36.1 ±1.6	32.4 ±2.0	29.7 ±1.8	26.1 ±2.0	17.7 ±1.4	9.6
Total dry weight (mg). .	458.1 ±16	406.5 ±16	409.9 ±16	442.1 ±18	435.6 ±18	389.5
Mean value for nodule weight as percentage of whole plant weight . . .	7.9	7.9	7.2	5.9	4.1	2.5
Number of nodules . . .	90	69	59	61	64	54
Mean weight per nodule (mg)	0.40	0.47	0.50	0.43	0.28	0.18
N tops (% D.Wt.). . . .	3.28	3.77	3.83	3.70	4.06	5.28
N roots (% D.Wt.) . . .	2.22	2.32	2.28	2.28	2.61	2.85
N nodules (% D.Wt.) . .	4.38	4.77	4.85	4.96	4.92	4.91
N whole plant (% D.Wt.).	3.17	3.58	3.64	3.51	3.85	4.90
N per plant (mg)	14.53	14.55	14.91	15.51	16.97	19.12

The number of nodules decreased only slightly at the higher urea concentrations, presumably because many nodules had already been formed when the first sprayings were carried out. The mean weight per nodule increased at the lower urea concentrations, but decreased markedly at concentrations of 4 and 8%. The nitrogen

content of the tops and roots and even that of the nodules was increased by the sprayings. The total uptake of nitrogen per plant increased with increasing urea concentration.

DISCUSSION

In the experiments with white clover and lucerne the most outstanding observation was that the formation of nodules in clover was increased by various concentrations of glucose and sucrose, both in the absence and presence of additional light, whereas nodule formation in lucerne was only promoted by 0.2 and 0.5% sucrose in the first period of growth, and was ultimately checked by all concentrations of glucose and sucrose, not only in the presence but also in the absence of additional light. This suggests that the intensity of the artificial light was insufficient for the clover plants, *i.e.* white clover plants need more light for maximum nodule formation than do lucerne plants. It may be expected that with increasing light intensity, nodulation will be checked sooner in lucerne than in white clover, as nodule formation in lucerne was ultimately depressed even by low concentrations of carbohydrates in the growth substrate. Since the additional light has stimulated nodule formation in lucerne, it is not clear why low concentrations of carbohydrates did not favour nodulation in the absence of additional light during the whole growth period of these plants. As it seems unlikely that in the absence of additional light, the C/N ratio in the lucerne plants has risen to an abnormally high level in the presence of 0.2% glucose or sucrose, the only explanation would be that the carbohydrates in the medium have also exerted a local (negative) effect on nodulation in these plants.

The depressing effect of too low a light intensity on the number of nodules in the pea plants was not counteracted by spraying with sucrose solution. This is in accord with the finding of McGonagle [24], that addition of sugar to the medium did not stimulate nodulation in field peas grown in the dark. Moreover, the reduction in the number of nodules by supplying combined nitrogen in the presence of additional light has not been counteracted by spraying with sugar solution. It is also remarkable that whilst the addition of combined nitrogen has increased the weight of those plants grown with additional light which had not been sprayed, this did

not happen with the plants which had been sprayed with a sugar solution (compare Table 2 with Table 1).

In the second experiment with peas, it has been shown that the number and total weight of nodules decreased when the leaves were sprayed with urea solutions. Without any doubt, this effect has been exerted by the nitrogen present in the plant tissues. Although the lowest urea concentrations have not favoured infection, the concentrations of 0.5 and 1% have increased the size of nodules; treatment with 4 and 8% urea solution, however, gave rise to a reduction in the size of nodules.

In contrast with the former experiment, in which plant weight was stimulated by combined nitrogen when the plants were grown with additional light and not sprayed with a sugar solution, the provision of the plants with urea nitrogen sprayed on the leaves has not increased the weight of the plants, although the seeds had been inoculated with the same strains of *Rhizobia*. The top/root ratio and the nitrogen content of the roots have been only slightly increased by spraying with 0.5, 1 and 2% urea solution. Nevertheless, the number of nodules has been distinctly decreased by these treatments. A decrease in the size of nodules was correlated with a high top/root ratio and a high nitrogen content of the roots. Probably this was due chiefly to lack of carbohydrates in the roots, or to a low C/N ratio in the roots. It is very likely that the ratio of soluble nitrogenous compounds to soluble carbohydrates in the plant tissue had been increased by spraying with urea solutions and that this has affected the production and size of the nodules. In this connection it is of interest to note that tuber formation in potatoes, grown aseptically in the dark, is also influenced by the proportion of soluble carbohydrates to soluble organic nitrogen in the plant tissue [33].

From the data reported by several workers it seems very likely that too low as well as too high a ratio of soluble carbohydrates to soluble nitrogenous compounds will be harmful for nodulation in legumes.

It is highly probable that nodulation is not only internally influenced by the C/N ratio within the plant, but also externally, by carbohydrate and nitrogenous compounds in the medium.

Reviewing the whole subject, dealt with in this paper, it must be concluded that much remains still unsolved and many controversial opinions must still prevail.

SUMMARY

Experiments were carried out in winter with white clover and lucerne which had been inoculated and grown aseptically in agar in the absence of combined nitrogen. The effect of various concentrations of glucose and sucrose on nodulation was studied in plants grown in the presence and in the absence of additional light (15 hours per day).

In the first period of growth especially, concentrations of 0.5 and 1% glucose stimulated nodule formation in clover plants, both in the absence and presence of additional light; ultimately, the highest numbers of nodules were formed in the presence of 2% glucose. In the absence of additional light, nodulation was stimulated by 0.5, 1 and 2% sucrose and in the presence of additional light, by 0.5 and 1% sucrose; in both cases, 0.5% sucrose effected the greatest stimulation. Nodulation of white clover plants was checked more by high concentrations of sucrose than by high concentrations of glucose.

In contrast with the clover plants, nodulation in lucerne was already checked by low concentrations of glucose, even of 0.2%. In the presence or absence of additional light, a stimulation of nodule formation was only observed in the early period of plant growth, by 0.2 and 0.5% sucrose. Nodulation in lucerne was delayed for several weeks by 2% sucrose and checked entirely by 5% glucose or sucrose.

In a second experiment, inoculated peas were grown aseptically in quartz sand in Weck glass jars, in the absence or presence of combined nitrogen. In half the cultures the effect was studied of five successive sprayings of the leaves with 0.5% sucrose solution at intervals of 3 to 4 days. Within each treatment half the cultures received additional artificial light. It has been shown that the depressing effect of too low a light intensity on weight of nodules and nitrogen fixation may be partially overcome by spraying the leaves with a sugar solution. However, the reduction in the number of nodules by a lack of light or by addition of combined nitrogen has not been counteracted by spraying with a sugar solution. In a second experiment with peas, the influence has been studied of various concentrations of urea solution sprayed on the leaves at intervals of four to five days. The number and total weight of nodules were decreased by this treatment. The size of the nodules was increased by concentrations of 0.5 and 1 % urea, but decreased by concentrations of 4 and 8%. A decrease in the size of nodules was correlated with a high top/root ratio and a high nitrogen content of the roots.

It is highly probable that nodulation is not only internally influenced by the C/N ratio within the plant, but also externally by carbohydrate and nitrogenous compounds in the medium.

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APPENDIX

(referring to footnote, p. 94)

After this paper was submitted for publication a paper by Krasnikov *et al.* appeared in Mikrobiologiya **27**, 599–604 (1958). A marked positive correlation was found to exist between the monosaccharide content of the roots and the intensity of nodulation in different varieties of peas and beans. A less pronounced positive correlation was noted between the monosaccharide content of the green parts and nodulation. The polysaccharide content correlated negatively with nodulation.

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FERTILIZING OF YOUNG OIL PALMS IN THE CAMEROONS

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INTRODUCTION

A considerable amount of work on nutrition of the oil palm (*Elaeis Guinensis* Jacq.) in West Africa and the Belgian Congo is in progress [2 3 4 5 6 7 10 12 13].

As part of the investigations on the use-capabilities of the land leased to the Cameroons Development Corporation, which is situated on the glacis of the Cameroon Mountain range, a fertilizer trial was laid out in young oil palms, planted in 1955 in an area of old oil palms in M'Pundu – Ekona.

Prior to it some preliminary information had been obtained on the growth responses of oil palm seedlings to nitrogen, phosphorus and potassium in the nursery.

The present study on the effect of the main plant nutrients nitrogen, phosphorus, potassium and magnesium is based upon observations made on growth and flowering and on chemical analysis of the leaves.

METHODS

1. *Nursery experiment*

The experiment was of a randomised block design with the following treatments: control (0), nitrogen (N), phosphorus (P), potassium (K) and their combinations NP, NK, PK, and NPK, in four replications, two of which received a dressing of magnesium (Mg).

Palm seedlings, germinated in May 1954, were planted in baskets a month later.

Each plot held 12 seedlings and was surrounded by a guard row. The baskets were filled with equal weights (5 kg) of topsoil from a nearby oil palm plantation.

Rockphosphate (30 to 32% P_2O_5) at the rate of 10 g per basket was mixed with the soil filled in the phosphate-treated baskets. Sulphate of ammonia and muriate of potash were applied monthly at the rate of 6 and 3 g respectively per month. Sulphate-of-magnesium dressings were made monthly at the rate of 1 g per basket.

The experiment was discontinued ten months after planting, *i.e.* in March 1955. Growth responses were assessed visually.

2. *Field experiment*

Nine months old Tenera oil palm seedlings originating from one controlled pollinated bunch were selected in a plantation nursery, so as to be of a similar size and uniform in appearance. They were planted in an area of about 22 acres, carefully surveyed for uniformity of soil. The soil in the top 30 cm was a clay-loam with fine to medium sand (Munsell colour: 10 YR 6/3), well drained with a few faint fine orange mottles, gradually changing to a sandy to very sandy clay down to 130 cm (Munsell colour: 7.5 YR 5/8) with common distinct fine orange mottles at 100 and at 130 cm changing into few to common fine distinct blue mottles. The pH was 6.5 in the top 20 cm, decreasing to 6.0 at 20 cm and 5.0 at 40 cm. Calcium carbonate content was estimated at 0.5% in the top 30 cm but it decreased with depth until there was none below 30 cm.

The average annual rainfall was approximately 90 inches, with a dry season of three months (less than 4 in. per month) extending from the middle of November to the middle of February.

Proper attention has been given to the establishment and maintenance of Pueraria cover crop.

The design is a 2^4 factorial with four replications of eight plots each, nitrogen, phosphorus and potassium being at two levels (0 and 1) and the main effect of magnesium confounded with the replications, two of which had a dressing of magnesium.

Fertilizers were applied at the following rates per annum (ounces per palm):

Year	Sulphate of ammonia	Rockphosphate	Muriate of potash	Magnesium sulphate
1955	8	4	10	8
1956	12	6	16	12
1957	24	12	32	24

Two applications of fertiliser were made, one half during the period of the early rains (April) and the other during the period of the late rains (October) The first application, however, was made in June 1955 at the time of planting.

Each plot of 16 trees was surrounded by a single guard row, the planting density being 30 ft. triangular.

Every six months the length of the first fully opened leaf on each palm was measured and an average figure obtained for the 16 trees in each plot. The first fully opened leaf was considered to be the youngest fully developed leaf, contrasting with the spear leaf. The first measurements were made in December 1955. Since June 1957, at the onset of flowering, records were kept monthly on the number of male and female inflorescences on each palm.

For the determination of potassium, phosphorus, magnesium and calcium in the leaves, bulk leaf samples were taken monthly from each plot of 16 palms and analysed, in accordance with the methods described by Broeshart [1 2 3 4]. Sampling began in February 1957 but as laboratory analysis did not begin until November of the same year, data are only available for the months of February, March and April 1957.

RESULTS AND DISCUSSION

1. NURSERY EXPERIMENT

Growth response was first observed in the NPK and NP plots three months after the first fertilizer application.

At the end of March 1955, that is after 10 months, the experiment was discontinued and a series of photographs taken, two of which are reproduced (Plates 1 and 2).

Plate 1 shows that of the single fertilizer dressings. N produced the largest response in leaf growth. Combination of N with P and/or K had a slightly greater effect than N alone in the descending order NPK, NK, NP and N.

Plate 2 shows that P had at least as favourable an effect on root development as N. There was no response to Mg dressings.

2. FIELD EXPERIMENT

A. *Leaf length*

The results from the leaf length measurements taken every half year from December 1955 until December 1957, *i.e.* $^1/_2$, 1, $1^1/_2$, 2, and $2^1/_2$ years after planting, are presented in Table 1. The mean leaf length (expressed as a percentage of the control) for the different treatments at increasing ages of the palms and the main effects and interactions between the nutrients are shown.

Although no significant differences appear in the first half year of growth after planting (from June 1955 to December 1955),

TABLE 1

Treatment	Leaf length (expressed as percentage of control)									
	December 1955		June 1956		December 1956		June 1957		December 1957	
	L.L. †	M.E. †	L.L.	M.E.	L.L.	M.E.	L.L.	M.E.	L.L.	M.E.
O	100 (= 85.0 cm) ‡		100 (= 97.0 cm)		100 (= 144.5 cm)		100 (= 191.5 cm)		100 (= 245.0 cm)	
N	104	59	106	180 **	109	148 **	110 *	240 **	106	218 **
P	98	—3	96	30	98	24	98	24	96	—42
K	102	—11	103	60 *	104	168 **	108	178 **	107	288 **
NP	105	—19	102	2	103	—16	105	—16	103	—22
NK	98	—107*	110	—46	109	—86 *	107	—154 **	107	—188 *
PK	103	39	92	10	107	70	108	102 *	108	—40
NPK	100	11	112 *	48	113 *	26	112 *	86	107	—16
Mg	89	—35	88	0	91	—34	93	22	95	170(confounded)
NMg	115	45	117 *	34	110 *	24	116 **	90	110	98
PMg	90	—9	91	—44	93	12	96	38	100	22
KMg	97	—1	102	—2	107	—28	106	16	113 *	36
NPMg	102	—37	108	—56	104	—28	109	—26	113 *	—30
NKMg	95	—45	107	—96 **	103	—86 *	108	—60	117 **	—56
PKMg	103	29	104	20	108	2	110 *	16	110	—116
NPKMg	98	21	107	—10	111 *	18	116 **	24	111	12
	Significant differences (L.L.) and levels (M.E.)									
$P = 0.05$	17.4	84	11.0	60	9.7	79	9.3	101	11.2	156
$P = 0.01$	24.1	116	15.3	84	13.4	110	12.9	140	15.5	217

† L.L. = Leaf length (% of control); M.E. = Main effects and interactions.
‡ Absolute leaf length.
* = Significant at probability of 5%.
** = Significant at probability of 1%.

Plate 1. Effect of fertilizing on oil palm seedlings.

Plate 2. Root development of oil palm seedlings shown in Plate 1.

treatments with N, and especially treatment NMg (nitrogen plus magnesium), seem to be favourable.

The interaction between N and K is the only one that reaches a significant (negative) level.

After one years' growth, the indications from the previous measurement are substantiated, treatments NPK and NMg being significantly better than control. Also the main effects of N and K and the negative interaction N × K × Mg attain a significant level.

Table 1 shows that the level to reach significance at P 0.05 is approximately 84 for December 1955 and 60 for June 1956. This suggests that growth responses are more variable in the first half year, although leaf lengths are smaller, than after one year when the plants have overcome the transplanting shock and settled down. In other words, to attain statistical significance a relatively larger percentage increase is required in December 1955 than in June 1956. As an indication of irregularities in growth and growth response, this observation is useful and will be dealt with again later.

The trend in December 1956 is principally the same as in the previous measurements; that is, outstanding growth responses from fertilizing with N or K, and poor response to NK dressings. Besides treatments NMg, only the NPK treatments are significant, which emphasizes the importance of P in enabling the plant to make full use of NK dressings. A positive and significant second-order interaction between N, P and K would provide definite proof for this indication. As may be seen in Table 1 such a level is not reached.

The marked response of the palms to NMg dressings is not limited to the first measurement alone so that the exceptionally high fertility of the NMg plots occurring by chance as suggested by the December 1955 figure, may be excluded. Positive interactions between N and Mg are known in other crops. Indications of a similar effect in oil palms may explain the ultimately unsatisfactory situation in respect of the accepted observation that N alone has generally no effect on oil palms in West Africa and the Belgian Congo [11].

The results differ in so far that, at least in young palms, even with a well-developed leguminous cover crop, N does have a marked effect on growth. The interaction N × Mg however, does not attain a level of significance in any of the individual, half-yearly analyses.

In view of a possible Mg deficiency, it could be argued that the negative interaction N × K is not due to P deficiency, as suggested before. In that case, however, one would not expect a negative interaction N × K × Mg as observed in June and December 1956 (Table 1). Moreover, a response to P is supported further by the positive interaction between P and K obtained in June 1957, showing also that it is in the P–K relationship that P plays its role rather than in the N–P balance. A similar tendency has been noticed in current banana and rubber fertiliser trials.

The trends in the last two measurements (June and especially December 1957) are markedly different from the previous ones.

Although the main effect of Mg is confounded with the replications and consequently cannot be assessed, it is remarkable that its variance rises steeply in the last half year, *i.e.* from June to December 1957 (Table 1)from 22 to 170, and since December 1955 from –35 to 170.

From Table 1 it may be seen that in the last period the rise in levels of significance is disproportionally high, which as mentioned before provides an indication of irregularities in growth or perhaps growth response to fertilizer application. Although the same main effects and interactions are still significant, it is noteworthy that neither the NPK nor the NMg treatments are any longer significantly different from the control. In their place appear three treatments with Mg, that is NPMg, NKMg and KMg.

In the following paragraphs these results will be related to observations made on flowering, the incidence of Crown Disease [6], and the chemical composition of the leaves.

B. *Flowering*

The first inflorescences were observed in June 1957, *i.e.* two years after planting.

On the 10th of June the trees that had flowered, were counted (Table 2).

When correlating the number of trees which had flowered before the 10th of June 1957 with the length of the leaves in the same month, a highly significant (P 0.01) coefficient of 0.697 is obtained. It appears that the length of the first fully developed leaf is a good indication of the growth of the young vegetative palms.

Analysing the results of Table 2 with the χ^2 test, a highly signifi-

cant departure from homogeneity is found. Checking the departure from the average flowering incidence, it appears that control and treatment P are highly significantly inferior, whereas NPK dressings are highly significantly superior. Evidently, all fertilized trees, except those dressed with P alone, have a shorter vegetative period of growth, treatment NPK being outstanding.

TABLE 2

Palms flowered before 10th June 1957		
Treatment	No. of palms	
	Flowered	Not flowered
O	3	61
N	26	38
P	7	57
K	26	38
NP	27	37
NK	23	41
PK	21	43
NPK	29	35

Analysis of the main effects and interactions gives results similar to those demonstrated for leaf length, *i.e.* significant main effects of N and K, and a negative interaction between them (significant at the 1% value for χ^2).

Analysing the effect of Mg on the length of the vegetative period, gives the interesting result that it increases the number of trees having flowered before the 10th of June from 26% to 37.5% (Table 3).

TABLE 3

Palms flowered before 10th June 1957			
Treatment	No. of palms		
	Flowered	Not flowered	
Magnesium	96	160	χ^2: 8.12
No magnesium	66	190	(*P* 0.01: 6.635)

The total number of inflorescences (male and female) produced in the six months from June to November (inclusive) 1957, is represented in Table 4A.

It may be seen from Table 4A, that the main effects of N and K are significant. The negative interaction N × K is large but not significant. Although confounded, it is worth noting the large main

effect of Mg, which just fails to reach a level of 5%-probability.

In Table 4B the mean sex ratio of the inflorescences produced in the first six months of the reproductive stage (vide Table 4A) is shown.

The main effect of P and the interaction N × K × Mg are both negative and significant. Of special note is the positive interaction N × P × K × Mg, which is near enough significant at a probability of 5%. This together with the high sex ratio for the control plots, strongly indicates the importance of a balanced fertilizer.

TABLE 4

Treatment	A Total No. of inflorescences June – December 1957		B Sex ratio $\frac{\text{female inflorescences}}{\text{total inflorescences}}$ %	
	Total No. of flowers	Main effects and interactions	Sex ratio	Main effects and interactions
O	132		48.5	
N	299	881	26.0	55
P	133	77	16.0	—171
K	263	637	17.5	19
NP	242	—159	20.0	9
NK	280	—383	33.5	33
PK	198	225	9.0	95
NPK	356	113	23.5	23
Mg	145	435	14.0	—25
NMg	362	—21	41.0	7
PMg	175	167	15.0	57
KMg	241	55	24.5	89
NPMg	314	—325	12.0	—91
NKMg	360	—181	20.5	—163
PKMg	393	91	24.5	—15
NPKMg	348	—285	25.0	135
	Significant effect: at *P* 0.05 : 499 at *P* 0.01 : 692		Significant effect: at *P* 0.05 : 136 at *P* 0.01 : 188	

Growth responses to P dressings could not be demonstrated in the leaf length measurements except in the presence of additional N and K. The results from Tables 2, 3 and 4A give the same evidence and moreover point to a favourable effect of Mg in the period of transition from the vegetative to the reproductive stage.

To summarize, it may be concluded that the fertility of the experimental area is low but, at this low level, is apparently (Table

4B) well balanced. Increasing the productivity of this area by means of fertilizing, involves firstly applications of either K or N alone (or perhaps N with Mg). A further increase cannot be obtained from dressings of both N and K, unless P is added, thus NPK. This, at least, appears to be valid until the time the young oil palms enter the reproductive stage.

At the onset of flowering, the leaf measurements as well as the observations made on flowering suggest an increased need for Mg of the palms. In the field this was already observed earlier, in particular at Idenau, where in a 4000-acre plantation, replanted at the rate of approximately 500 acres per annum since 1950, at this critical period severe Mg-deficiency symptoms (7) were observed, which however disappeared again the next year, whether dressed with Mg or not.

In order to provide more evidence for the indication of increased Mg requirements of young oil palms, when they enter the reproductive stage, the effect of Mg on the leaf length was analysed over the entire period of observation. This was done by comparing the difference in leaf length between Mg and non-Mg dressed blocks at different ages of the palms (Table 5).

TABLE 5

Difference in leaf length between Mg and non-Mg dressed blocks	
Date	Mean difference in leaf length (in cm)
December '55	—2.19
June '56	0.00
December '56	—2.12
June '57	1.38
December '57	10.50

It appears that a pronounced change takes place in the period June to December 1957, which coincides with the appearance of the first inflorescences and the first occurrence of Crown Disease (vide paragraph C).

By grouping of age comparisons [10] a significant effect (P 0.05) is obtained for the difference in leaf length between Mg and non-Mg dressed blocks, *i.e.* when comparing the combined effect of June and December 1957 (reproductive stage) with that of the previous

three measurements (December 1955, June and December 1956), *i.e.* the vegetative period.

Another way of analysing the results is by resolving the age × Mg variance among the two physiologically different periods and weighing it against the block × age × Mg variance.

This statistical approach also provides a significant effect (P 0.05), showing that oil palms, when they enter the reproductive stage, demand more Mg.

C. *Crown Disease*

Crown Disease has been described by Bull [6] as a disease of unknown cause which generally affects the growth of young oil palms during their second and third years.

A severe outbreak of Crown Disease occurred in this experiment in July 1957. Two months later, at the beginning of September, 122 out of the 512 trees recorded were affected. Towards the end of the year most of them had recovered and only a few new cases were observed.

In Table 6 the distribution is shown of the number of cases in the different treatments on the 9th of September and the total number recorded in the period from July to December (inclusive) 1957.

TABLE 6

Incidence of Crown Disease		
Treatments	No. of cases	
	At 9 September 1957	July – December 1957
O	8	10
N	12	12
P	12	14
K	27	30
NP	13	13
NK	15	15
PK	20	22
NPK	15	21
With Mg	50	55
Without Mg	72	82

Tested with the χ^2 test, there appears to be a highly significant departure from homogeneity (χ^2 are 22.71 and 24.95 for column 1 and 2 in Table 6 respectively, whereas the P 0.01 value is only 18.48)

In both counts, treatments K show a very significant higher disease incidence, whereas the control plots have a much lower rate than the average for the experiment. Hence, it may be concluded that, in general, fertilizing increases the disease incidence and in particular treatments with K (main effect of K is significant at P 0.01). Also significant (P 0.01) is the lower disease incidence in the Mg-dressed blocks.

These results corroborate the conclusions drawn from the observations made on flowering and leaf length and demonstrate once more that the period of transition from the vegetative to the reproductive stage is a critical one.

In this respect, it is of interest that deficiency symptoms of N and Mg were not observed. K-deficiency symptoms as described by Bull [7], *i.e.* Confluent orange spotting was not evident, but the symptoms as described by Broeshart, Ferwerda, and Kovachich [5] were observed in most of the non-K and some of the K-treated oil palms, in particular in the blocks which received Mg.

The relationship between Crown Disease incidence and fertilizer treatment, together with the first outbreak at the onset of flowering, strongly suggests a physiological nature of the disorder: the more balanced the nutritional medium, the lower the incidence of Crown Disease.

D. *Discussion*

It was demonstrated in the preceding paragraphs that, although Mg increased the number of flowering trees and hence reduced the length of the vegetative period, it did not have an effect on leaf length before June 1957, whereas on the other hand a highly significant correlation ($r = 0.697$) was obtained for the whole experiment between leaf length and trees flowered before 10th of June 1957. This together with the finding of a reducing effect of Mg on incidence of Crown Disease warranted the conclusion that the incidence of Crown Disease was a reflection of a physiological disorder at first flowering of the palms, which was more severe the more unbalanced the growth medium at that particular period. The increased need for Mg at the time of first flowering must have affected those palms whose growth was stimulated by dressings of nutrients sub-optimal for vegetative growth.

This has been found to be true in respect of K (both as a treatment

and its main effect). It did not affect the N-treated palms, where an improved growth and hence a more unbalanced medium at flowering did not correspond with a higher disease incidence.

In order to give a satisfactory explanation for these conflicting results, it is clear that in view of the antagonism between K and Mg, K had a markedly unfavourable effect on Crown Disease. In respect of N, the results of Table 1 gave an indication of a possible positive interaction between N and Mg, or to put it in other words, N-dressed plants may be in a position to make better use of Mg, whether applied or available in the soil.

TABLE 7

Analysis of 5 half-yearly measurements combined December 1955 – December 1957 (inclusive)		
Treatment	Leaf length (% of control) average of 5 periods	Main effects and interactions
O	100 (= 152.6 ems)	
N	106.4 *	845 ***
P	96.9	—41
K	105.9 *	683 ***
NP	103.5	—59
NK	106.9 **	—581 ***
PK	105.1 *	181
NPK	109.3 ***	177
Mg	92.2 **	123 (confounded)
NMg	112.9 ***	291 **
PMg	95.3	89
KMg	106.9 **	—61
NPMg	108.4 ***	—165
NKMg	108.2 **	—343 **
PKMg	107.9 **	—49
NPKMg	110.2 ***	—87

* Difference or effect significant at $P = 0.05$
** ,, ,, ,, ,, ,, $P = 0.01$
*** ,, ,, ,, ,, ,, $P = 0.001$

In Table 7 the results of the individual half-yearly leaf length measurements are analysed as a whole. It may be seen that a significant positive interaction exists between N and Mg, which confirms the indications noticed in the individual analyses (Table 1) and may explain the different behaviour of N in respect of the incidence of Crown Disease.

The highly significantly smaller leaf length of the plots treated with Mg alone, shows that Mg dressings are most effective in

combination with other nutrients, and in particular with N. Of particular note is the change from a positive to a negative effect by PK and PKMg on leaf length between June 1957 and December 1957 in the absence of N (Table 1, for PK from 102 to -40 and for PKMg from 16 to -116). A covariance analysis of the results of these two periods fails however to attain significance.

TABLE 8

Analysis of 4 half-yearly measurements combined June 1955 – June 1957 (inclusive)		
Treatment	Leaf length (% of control average of 4 periods	Main effects and interactions
O	100 (= 129.5 ems)	
N	106.8 *	627 ***
P	97.6	1
K	105.3 *	395 ***
NP	103.9	—27
NK	106.6 *	—393 ***
PK	103.8	221 **
NPK	110.3 ***	193 *
Mg	91.0 ***	—47 (confounded)
NMg	114.1 ***	193 *
PMg	93.4	77
KMg	104.0	25
NPMg	106.4 *	—125
NKMg	104.2	—287 ***
PKMg	106.9 *	67
NPKMg	110.0 ***	75

* Difference or effect significant at $P = 0.05$
** ,, ,, ,, ,, ,, $P = 0.01$
*** ,, ,, ,, ,, ,, $P = 0.001$

This method of pooling the individual half-yearly measurements in one analysis does not bear out the expectation of a significant positive interaction between N, P, and K. Having gained a more detailed knowledge on the reactions of palms of different age, another variance analysis was worked out, *i.e.* of the observations up to June 1957, that is for the vegetative period only.

It may be seen from Table 8 that the second order interaction N × P × K attains a significant level, confirming that P plays a role in the nutrition of the vegetative palms in this experiment. The absence of an effect when including the results of December 1957 (vide Table 7), may be due to the larger variation resulting

from the unbalanced condition of the palms when entering the reproductive stage. It is also possible that in view of the changed requirements of flowering palms, the role of P is a different one from that for optimal growth as already demonstrated for Mg. The near enough significant interaction between N, P, K and Mg (Table 4B) however, does not favour this possibility. Future results may elucidate it, particilarly those from foliar analysis (vide paragraph E).

E. *Foliar analysis*

At the time of the preparation of this paper results of foliar analysis were available for the months of February, March and April 1957 only.

Optimal values for the leaf-nutrient contents, according to Broeshart [2], are as follows:

Nutrient	Percentage of dry matter
K_2O	2.05 – 2.30
P_2O_5	0.48 – 0.53
MgO	0.42 – 0.58
CaO	0.77 – 0.91

The averages (per cent of dry matter) for the experiment are:

	K_2O	P_2O_5	MgO	CaO
February	1.43	0.32	0.43	0.62
March	1.55	0.41	0.43	0.55
April	1.67	0.58	0.42	0.59

For the period under observation, these data indicate consistent deficiencies for K and Ca, while Mg is consistently marginal. The position of P seems to be somewhere between deficient and marginal.

The combined effect of three or four simultaneous deficiencies is rather unpredictable [2 4], and consequently the interpretation of the analysis is difficult. For the April analytical data, the effect of fertilizer dressings of N, P and K on the leaf nutrient contents has been assessed (Table 9). Optimal ratios for K_2O/P_2O_5 and K_2O/CaO, mentioned in the literature [2 8], are 3.40 and 2.65, respectively.

It would seem from Table 9, that fertilizer dressings have resulted in a few major and quite a number of minor changes in the leaf nutrient composition.

TABLE 9

Treatment	Leaf nutrient content (% of dry matter)											
	K_2O		P_2O_5		MgO		CaO		K_2O/P_2O_5		K_2O/CaO	
	%	M.E. *	%	M.E. *	%	M.E. *	%	M.E. *	ratio	M.E. *	ratio	M.E. *
O	1.65		0.55		0.49		0.71		3.00		2.32	
N	1.53	—0.07	0.57	—0.07	0.46	0.00	0.58	—0.02	2.68	—0.63	2.64	0.00
P	1.63	—0.04	0.60	—0.01	0.39	—0.02	0.54	—0.02	2.72	—0.45	3.02	0.04
K	1.85	0.13	0.61	0.03	0.40	—0.04	0.59	—0.04	3.03	1.51	3.14	0.41
NP	1.41	—0.04	0.58	—0.07	0.43	0.01	0.63	0.02	2.43	—0.13	2.24	—0.20
NK	1.86	0.00	0.59	—0.07	0.39	0.00	0.52	—0.01	3.10	0.59	3.58	0.11
PK	1.90	0.00	0.59	—0.13	0.37	—0.01	0.56	0.01	3.15	0.61	3.39	—0.03
NPK	1.65	—0.02	0.54	—0.01	0.38	—0.01	0.49	—0.03	3.06	—0.19	3.37	0.09
Mean	1.68		0.58		0.40		0.58		2.90		2.96	

* Main effects and interactions.

Although as yet too little information is available to interpret the results in detail, K is seen to have a marked effect. The main effect of K is significant, which means that the K_2O content has been increased from 1.62 to 1.75. Compared with Broeshart's data, it would seem that the potash dressings were quite insufficient to remedy the K deficiency. This is indeed likely in view of the observed deficiency symptoms, even in the K-dressed plots.

On the whole, the leaf nutrient composition agrees well with the observed growth responses, such as the comparatively high MgO content of the leaves in the control and N plots (vide Table 4B, sex ratio and Table 6, Crown Disease).

Correlation of the mean K_2O content in April 1957 and the mean leaf length per plot in June 1957, after having been adjusted for the main effects of nitrogen and magnesium, gives a significant coefficient of 0.35. In view of substantial first- and second-order interactions, which have an effect on leaf length, and also the complication of the multiple effect of three or four deficiencies, it is remarkable that the correlation coefficient, however small, attains the 5% level of significance.

SUMMARY AND CONCLUSIONS

The interrelations of nitrogen, phosphorus, potassium and magnesium have been investigated in young Tenera oil palms.

They were assessed visually in a nursery experiment with seedlings grown in baskets during ten months. In a subsequent field trial observations were made on growth, flowering, incidence of Crown Disease and chemical composition of the leaves. The following conclusions were drawn:

1. Careful selection of the experimental area on uniformity of the soil and of as genetically homogeneous planting material as possible has resulted in a remarkably low intra-class variance – treatment differences of an order of magnitude of 10% only attained significance as determined on a probability of less than 0.05.

As a result the multiple effects of the four tested nutrients, N, P, K and Mg, on growth response of vegetative and reproductive oil palms could be interpreted in detail.

2. Vegetative oil palms responded to dressings of N and K, but neither to P, unless in the presence of K or NK, nor to Mg, except in combination with dressings of N alone.

3. Vegetative oil palms, when they enter the reproductive stage, had a greatly increased need for Mg, in the absence of which an adverse effect on leaf length, hence growth, was noticeable.

4. The coincidence of the occurrence of Crown Disease and the transitional period from the vegetative to the reproductive stage in young oil palms has been studied in the light of a possible causal relationship with the increased need for Mg.

It has been demonstrated that Mg deficiency, resulting from increased Mg requirements of oil palms flowering for the first time, increased the incidence of Crown Disease and at the same time suppressed growth of reproductive palms.

These results strongly indicated a causal relationship between Mg deficiency and Crown Disease, or more generally a physiological disorder resulting from a nutritionally unbalanced growth medium.

5. A balanced nutritional medium appeared to have a more pronounced effect on reducing Crown Disease and increasing the sex ratio of the inflorescences than on the total number of inflorescences produced in the first half year of reproductive growth.

It would seem that conditions for maximal growth were not necessarily the same for optimal growth and possibly maximal production.

6. The levels of optimal leaf composition, as defined by Broeshart [2], were found to agree well with the evidence obtained from growth responses, as measured by the length of the first fully opened leaf.

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THE OXIDATION OF PHLOROGLUCINOL AS A MODEL FOR HUMIFICATION PROCESSES *

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INTRODUCTION

In studying the mechanism of the formation of humic substances by chemical processes, emphasis is often laid on the oxidation of phenols, preferably in presence of nitrogen-containing compounds [2 6 27 37 39]. The reactions of those phenols that give by their structures some indications about the first oxidation products, like hydroquinone [3 5 7 8 23 28 29 38], pyrocatechol [3 9 38], pyrogallol [3 31] have been most extensively studied. Since not all naturally occurring phenols are readily oxidised to quinones, it seemed worthwile to investigate the oxidation of a phenol, such as phloroglucinol, which is easily oxidised, but which does not lead one to expect formation of *p*- or *o*-quinone.

Relatively little is known about the mechanism of the oxidation of phloroglucinol, although its oxidisability has been known from the time of its discovery [15]. Hlasiwetz found in this oxidation not only substances of lower molecular weight (like oxalic acid), but also amorphous, "moderartige" substances. Formation of humic compounds was found by Eller [4] only under rather vigorous conditions, or in the presence of ammonia, at high pH. Blix [1] and Kisch [17], however, found that phloroglucinol acts as a catalyst in oxidative de-amination of amino acids (contrary to Kar [16]), and from Saito's investigation [22], too, one might expect that in the oxidation of phloroglucinol products are formed, which are able

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to react with amino acids. Biochemical conversion of phloroglucinol to humic substances has been effected by Treccani-Benetti and Schiesser [36].

We studied the oxidation of phloroglucinol under conditions similar to natural ones, at a pH about 7. Previously published researches [32 33 34 35] led us to the conviction, that a free radical is formed as a first oxidation product, and that substances originate with a higher molecular weight. The investigations reported in this paper, amplify these results.

METHODS AND RESULTS

A. Oxidation products of phloroglucinol react with amino acids.

0.10 g phloroglucinol and 0.660 g L-asparagine were dissolved in 100 ml buffer solution (NaH_2PO_4 0.0050 M + Na_2HPO_4 0.0050 M). To inhibit growth of fungi, some HgJ_2 was added. After having been kept in air at room temperature during 5 days, the solution was examined as to changes in absorption spectrum (Beckman model DU spectrophotometer). The spectrum is drawn in Fig. 1, together with the spectra of some controls, missing one of the reagents, either phloroglucinol, asparagine, or oxygen. A rather strong absorption at 340 mμ was found, if all reagents were present. With tryptophane instead of asparagine similar results were found.

An analogous experiment has recently been reported by Rinderknecht and Jurd [24]. They found after 14 days' reaction a maximum in the absorption spectrum at 450 mμ, which was not found in our experiments.

B. The products of this reaction contain nitrogen.

0.10 g phloroglucinol, 0.660 g L-asparagine and 1.00 g $K_3Fe(CN)_6$ were dissolved together in 100 ml buffer solution (NaH_2PO_4 0.0050 M + Na_2HPO_4 0.0050 M). After having been kept at room temperature in an atmosphere of nitrogen during 2 days, the mixture was acidified with 1 ml 4 N H_2SO_4, and the reaction product was extracted from it with iso-amylalcohol (4 times 10 ml). On the residue, remaining on evaporating the iso-amylalcohol solution to dryness, we performed a reaction on nitrogen after Lassaigne [12]. The presence of nitrogen could be demonstrated, if both phloroglucinol and asparagine were present; a control containing no phloroglucinol gave a negative test. The same results were obtained in experiments with 0.200 g tryptophane instead of 0.660 g asparagine. If glycine, proline, or lysine were used as amino acids, the reaction was always negative, even when phloroglucinol was present.

C. The oxidation products originating from the reaction of phloroglucinol with $K_3Fe(CN)_6$ in the absence of amino acids, can

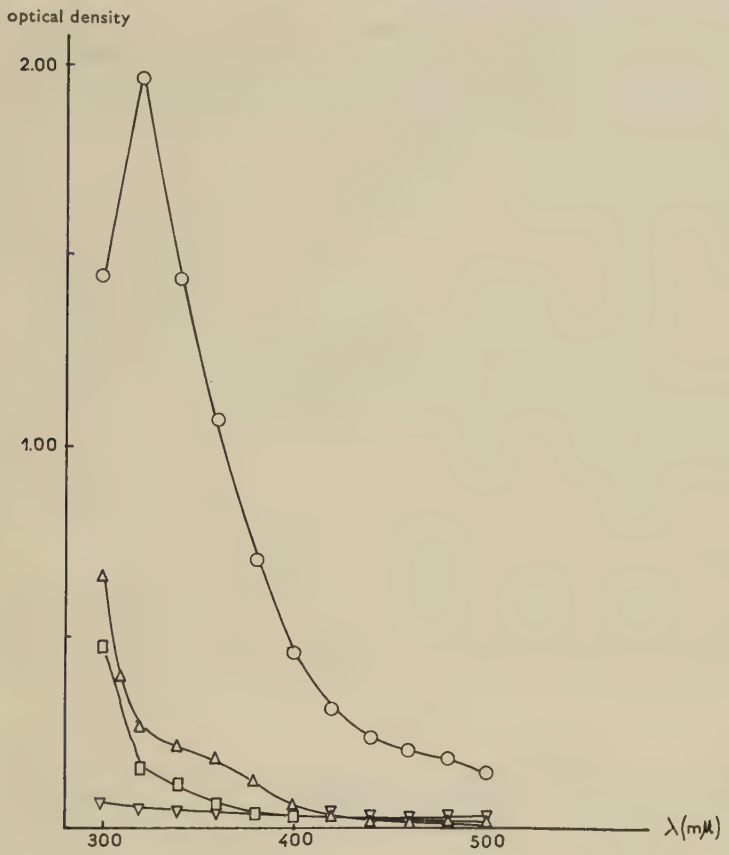

Fig. 1. Absorption spectrum of a solution originating on autoxidation of phloroglucinol in presence of L-asparagine, together with controls.
⊙: phloroglucinol + asparagine + O_2
△: phloroglucinol + asparagine
□: phloroglucinol + O_2
△: asparagine + O_2.

be separated by paper electrophoresis in 0.1 or 0.01 *N* NaOH. In addition to unoxidised phloroglucinol, three components could be detected. All oxidation products ran slower than phloroglucinol.

Electrophoresis was carried out on a strip of filter paper (TVN 47/58 *), whose breadth was 4 to 5 cm and whose length varied from 6 to 35 cm, according to the field strength desired. The paper was soaked in NaOH solution, which had been freed from dissolved oxygen by bubbling through purified nitrogen. The strip was rapidly dried by squeezing between two sheets of

* From N.V. Papierfabriek v/h G. Schut & Zonen, Heelsum.

filter paper. A mixture of oxidation products dissolved in 0.1 *N* NaOH was put on the paper, by drawing a line on it with a narrow-drawn capillary filled with solution of oxidation products. The strip of filter paper was placed between two glass plates in order to inhibit contamination of the solution by oxygen or CO_2 as much as possible.

The solution of oxidation products was prepared by extracting with 0.1 *N* NaOH all polyphenols from a solution in iso-amylalcohol, obtained as described in B.

The strength of current during electrophoresis varied from some tenths to some decades of mA; the time from some minutes to some hours.

In this way, the mixture of oxidation products is separated into 4 components, including unoxidised phloroglucinol (demonstrated by the colour reaction with vanilline-HCl, also isolated as described in Reference [33]). If we put the electrophoresis velocity of phloroglucinol equal to 1.0, the velocities of the other components are:

0.9 to 1.0: yellow brown colour in 0.1 *N* NaOH.
0.6 to 0.7: red purple colour in 0.1 *N* NaOH.
0.2 to 0.3: brown colour in 0.1 *N* NaOH.

The two last-mentioned components are only feebly visible.

In 0.01 *N* NaOH the same ratio of velocities was found.

In trying paper chromatography of the oxidation products in alkaline solution we found, that all oxidation products ran as fast as the liquid front, which excludes the possibility that the paper retards their electrophoresis.

D. The results, mentioned under C, indicate that the charge density of the oxidation products in 0.01 *N* NaOH (by which one might understand, for example, the average number of hydroxyl groups per benzene nucleus, dissociated in 0.01 *N* NaOH) is lower in the oxidation products than in phloroglucinol.

Electrophoresis in such concentrated NaOH solutions does not easily permit an exact theoretical treatment, particularly as we are dealing here with particles differring rather much from a stiff sphere. Nevertheless some qualitative indications might be drawn from the experiments.

For stiff, spherical particles one can derivate by using Robinson and Stokes' correction formulae [25], that the charge density in the oxidation products is lower than in phloroglucinol. Some insight into the applicability of these formulae may be gained by considering that, for the ratio of electrophoretic velocities of phloroglucinol and phenol in 0.1 *N* NaOH, experimentally 1.6 is found; neglecting any double layer interaction one calculates 1.88, while Robinson and Stokes' formulae give 1.73.

For stiff, non-spherical particles no deviations from the theory are to be expected if the particles are small as compared to the dimensions of the double layer [22], which applies to our experiments, at least to those carried out in 0.01 *N* NaOH, the double layers' extension as calculated according

to the Debye-Hückel theory being 6 to 10 times the particles' radius as calculated from diffusion experiments [33].

The influence of particle flexibility might be estimated by the following reasoning. If we neglect as a first approximation the influence of the double layers on the electrophoretic velocities, we get:

$$u = \frac{q.E}{f}$$

u = electrophoretic velocity
q = charge, $= z.V$
z = charge density
V = volume
f = friction; for a chain built from completely independent links proportional to V; by mutual interactions of the links less than proportional to V (Ref. [11]); for a stiff, spherical particle proportional to $\sqrt[3]{V}$. So we get $f \sim V^n$ with n varying between $1/3$ and 1.
E = field strength.

Now we can express our experimental results in the inequality

$$\frac{u_p}{u_w} = \frac{z_p \times V_p}{f_p} \times \frac{f_w}{z_w \times V_w} < 1.$$

In this formula, index p denotes quantities belonging to phloroglucinol, index w quantities belonging to oxidation products. Applying the formula for f:

$$\frac{z_p \times {V_p}^{1-n}}{z_w \times {V_w}^{1-n}} < 1.$$

And as $V_w > V_p$, which follows from diffusion measurements [34], while $n < 1$, we get:

$$z_w < z_p.$$

This derivation should, however, be regarded as a very rough approximation only, the neglection of the double layers' influence being probably unjustified.

Considering the uncertainties in the interpretation of the electrophoretic velocities, no more than an indication for the charge density of the oxidation products can be obtained.

CONCLUSION

When phloroglucinol is oxidised in neutral aqueous solutions by $K_3Fe(CN)_6$ in the presence of some amino acids, nitrogen-containing substances are found among the reaction products. If the oxidation is carried out in the absence of amino acids, the products presumably contain less dissociable hydroxyl groups per benzene nucleus than phloroglucinol.

For these phenomena, one can quote some parallel observations in nature: Forsyth [10 11] and Kukharenko [18] for example found, that with decomposing plant wastes, while the content of methoxyl groups decreases, the content of hydroxyl groups increases but not to an equivalent extent, some hydroxyl groups vanishing in the mean time. Mattson and Koutler-Andersson [19] found decreasing buffer capacity at a pH between 7 and 10 and increasing buffer capacity at a pH lower than 7, both on autoxidation and on humification of plant materials. Scheffer *et al.*, in an interesting paper [30], came to the conclusion that the genesis of humic acids is associated with increasing particle weight, but decreasing content of reactive groups. The uptake of nitrogen in decomposing plant wastes, similar to our experiments described in B, is well known[13 20 37].

Our investigation shows, that such processes may take place by purely chemical means even with a phenol that does not easily form quinones on oxidation. As such phenolic compounds are quite abundant in nature, they and their oxidation products, including radicals, should be considered in discussions on the mechanism of humification.

SUMMARY

With the oxidation of phloroglucinol, phenomena are observed reminiscent of humification processes. It is suggested, that in discussing humification more attention should be paid to the oxidation of phenols, which are not likely to give quinones when being oxidised.

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THE EFFECT OF GAMMA RADIATION ON BACTERIAL NODULE FORMATION

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Previous investigations by Sparrow and Gunkel[1] showed that tumor development was promoted by γ-irradiation, As bacterial nodules are morphologically similar to tumors, it was thought possible that γ-irradiation might also promote the formation of bacterial nodules in leguminous plants. If this would prove to be true it would be of considerable economic importance since nodule bacteria play a chief part in the nitrogen nutrition of leguminous plants and in supplying the soil with available nitrogenous compounds. In order to test whether γ-irradiation has such an effect two species of leguminous plants were tested namely, *Trifolium alexandrinum* and *Trigonella foenum-graecum*. Seeds of these plants were subjected to different dose rates (Table 1) from a radium tube for a period of seven days.

These experiments were repeated in two successive years, for confirmation. In addition to observations on bacterial nodules determination of the number of lateral roots, as well as of the number of clusters per plant, were made.

EXPERIMENTAL RESULTS

Effect of irradiation on bacterial nodules of Trifolium alexandrinum

Experiments were performed in two successive years, 1956 and 1957; seeds were exposed to different dose rates and planted. In the first year observations were made on the seedlings when they became 60 days old. In the second year the age of the seedlings was only 28 days. In both years plants exposed to high dose rates produced a greater number of bacterial nodules. The difference

between the treated (R_1 and R_2) and control plants was highly significant. In the experiments of 1956, the mean number of nodules per plant was 9.2 for plants exposed to a low dose of 427.0 r as shown in the figure. This number was nearly equal to that of the control (9.4). At a dose of 531.0 r the number of nodules showed a slight increase, amounting to 10.0 on the average. In plants exposed to a dose of 816.0 r the number of nodules reached a maximum value of 17.9, which is approximately double that of the control. At the dose 1855 r a slight decrease in the number of nodules was noticed, but it is still much greater than that of the control.

TABLE 1

Effect of gamma radiation on *Trifolium*

Treatment	Dose rate per day, r	Total dose, r	After 60 days (1956)		After 38 days (1957)		
			Mean no. of bacterial nodules per plant	Mean no. of lateral roots per plant	Mean no. of bacterial nodules per plant	Mean no. of lateral roots per plant	Mean no. of clusters per plant
C (Contr.)	—	—	9.4±3.1	17.4±4.2	6.6±2.1	11.7±3.4	0.86±0.19
R_1	265.0	1855.0	16.8±4.0	28.3±5.2	8.5±2.7	17.6±4.1	1.60±0.23
R_2	116.6	816.0	17.9±3.6	26.3±4.4	10.2±3.1	20.0±4.3	2.06±0.36
R_3	75.8	531.0	10.0±3.2	23.5±5.4	7.4±2,2	14.6±3.5	1.15±0.21
R_4	61.0	427.0	9.2±3.2	17.8±4.8	6.5±2.3	11.7±3.4	0.93±0.18

The curve representing the variation in the number of nodules under different irradiation intensities in the year 1957 follows approximately that of the previous year.

Effect of irradiation on bacterial nodules of Trigonella foenum-graecum

In the case of *Trigonella* irradiated plants also showed a greater number of nodules as compared with the control. It is evident from the curve representing the variation in number of nodules in the year 1956 that at the relatively low dose of 427.0 r the number of nodules (17.1) was slightly greater than the control (14.2). At a dose of 531.0 r a slight increase in the number of nodules was noticed A maximum number of 24.8 nodules was produced at a dose of 816.0 r. At a still higher dose of 1855 r a decrease in the number of nodules to 22.8 was recorded.

Again the curves found in 1957 follow the same trend as those from 1956. It is quite evident then, that there is coincidence in the

maximum number of nodules in both *Trifolium* and *Trigonella*.

The curve showing the variation in the number of nodules under different irradiation doses in the year 1957 runs more or less parallel to that of the previous year.

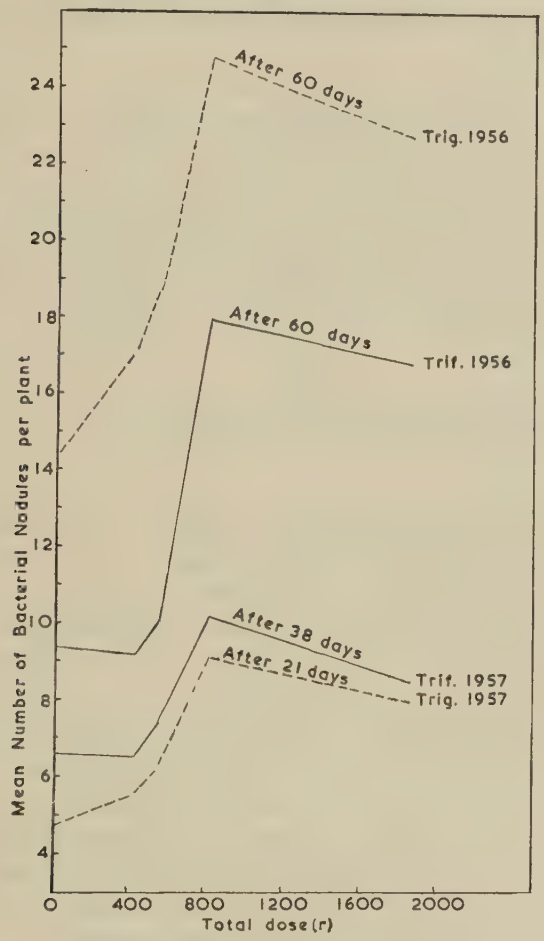

Fig. 1. Effect of γ-irradiation on bacterial nodule formation.

It was stated by Whyte *et al.* [2] that the effectiveness of the nodules is indicated by the presence of groups of large, pinkish, fleshy nodules of various shapes which are clustered in the vicinity of the crown and the first formed secondary roots. Examination of

the nodules of irradiated plants showed that they were pinkish in colour, and that the formation of clusters is more frequent in irradiated plants than in the control ones (Table 1 and 2). The highest degree of cluster formation in both *Trifolium* and *Trigonella* was observed in plants exposed to a dose of 816.0 r. These observations point to the effectiveness of nodules of the irradiated plants.

TABLE 2

Effect of gamma radiation on *Trigonella*

Treatment *	After 60 days (1956)	After 21 days (1957)		
	Mean no. of bacterial nodules per plant	Mean no. of bacterial nodules per plant	Mean no. of lateral roots per plant	Mean no. of clusters per plant
C	14.2 ± 2.9	4.7 ± 0.9	6.1 ± 1.8	1.28 ± 0.23
R_1	22.8 ± 4.1	8.0 ± 2.1	9.8 ± 2.4	1.95 ± 0.21
R_2	24.8 ± 4.2	9.1 ± 2.3	11.3 ± 2.9	2.37 ± 0.34
R_3	18.9 ± 3.1	6.2 ± 1.5	8.0 ± 2.7	1.48 ± 0.24
R_4	17.1 ± 3.4	5.5 ± 1.1	6.7 ± 1.9	1.28 ± 0.24

* For dose rates and total doses see Table 1.

Another interesting result is the increase in number of lateral roots within a certain range of irradiation doses. It is evident from Table 1 that in the case of *Trifolium* plants exposed to the relatively low dose of 427 r, had a mean number of lateral roots per plant approximately equal to that of the control. At higher doses a remarkable increase was noticed, particularly at a dose of 1855 r.

In another experiment, carried out in 1957 on *Trifolium* a similar behaviour was observed, but the maximum number of lateral roots was recorded at a lower dose of 816.0 r.

In the case of *Trigonella* the number of lateral roots produced under different irradiation doses bore the same relationship to the dose as in the case of *Trifolium*. At the relatively low dose the difference in the number of lateral roots between the irradiated and control plants was insignificant, whereas at higher doses, it was slightly significant. At a dose of 816.0 r the number of lateral roots (11.3) was approximately double that of the control (6.1).

Colchicine treatment was found to have the same effect as irradiation in regard to inducing the frequent formation of bacterial nodules and lateral roots. Seeds of *Trifolium* were treated with

colchicine (by soaking for 24 hours in a 0.5% solution) then planted and the growing plants examined after three and four weeks for the number of bacterial nodules, lateral roots, and frequency of clusters. The results revealed that after three weeks the number of nodules in the treated plants was about five times as much as in the control, while after four weeks it was more than three times as much (Table 3).

Wipf and Cooper [3] stated that infected cells of nodules of clover, vetch, and pea are regularly tetraploid. This may imply that the nodules develop from special, tetraploid, celles under normal conditions. From our knowledge about the effect of colchicine on plants it induces polyploidy in the somatic cells. It may be that in colchicine-treated plants, the frequent occurrence of nodules is due to the fact that colchicine treatment increases the number of tetraploid cells from which the nodules develop.

TABLE 3

Effect of colchicine on *Trifolium* and *Trigonella*

Treatment	After 3 weeks (1957)			After 4 weeks		
	Mean no. of bacterial nodules per plant	Mean no. of lateral roots per plant	Mean no. of clusters per plant	Mean no. of bacterial nodules per plant	Mean no. of lateral roots per plant	Mean no. of clusters per plant
Control	0.47±0.09	3.3±0.41	0.016±0.03	1.23±0.25	9.37±2.3	0.35±0.09
Treated	2.20±0.35	4.8±0.45	0.36 ±0.08	4.37±0.54	15.63±3.8	1.23±0.28

The clusters were more frequent in the treated than in the untreated plants. Also the number of lateral roots was larger in the case of the treated plants, particularly at the age of four weeks. It was 15.6 for the treated and 9.4 for the untreated plants.

The results presented in this paper are just a preliminary study in the field of the effect of irradiation on leguminous plants. In future years we hope to be able to extend this work in the direction of investigation the interrelations of irradiation dose, nodulation, growth, and yield of leguminous plants.

SUMMARY

Seeds of *Trifolium* and *Trigonella* were planted after exposure to different dose rates 61.0, 75.8, 116.6 and 265.0 r/day from a radium tube for a period of seven days. The general effect was a tendency towards increase in the

number of nodules and of lateral roots per plant, especially at a total dose of about 816 r.

Colchicine treatment was found to have a similar effect.

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ON THE DISTRIBUTION OF FUNGI IN THE RHIZOSPHERES OF OATS

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INTRODUCTION

The concept of soil as a mosaic of microhabitats colonised by micro-organisms is well known, but the factors which govern the patterns of distribution of such organisms are obscure and the precise patterns require closer study. The rhizosphere has received much attention particularly as concerns the bacterial component of this population. Despite the demonstration of quantitative changes in the rhizosphere microflora corresponding to changes in the gross age of plant roots (Starkey [14]) information of the fungal species complement of rhizospheres is seldom available. Much of the qualitative work has been directed towards demonstrating differences between the total rhizosphere population and the soil population (Timonin [16], West and Hildebrand [20], Katznelson and Richardson [4]). While Harley [1] divides the root region into the rhizosphere and the root-surface zones, and the work of Harley and Waid [2] on beech roots and of Waid [17] on rye-grass roots has provided detailed information of the zonation and succession of fungi on root surfaces, there is little comparable information of similar phenomena in the soil adjacent to roots – the rhizosphere zone. This paper seeks to provide some information of these topics for fungi in the rhizospheres of oats.

METHODS

An assessment of methods available for the isolation of fungi from a rhizosphere has already been given (Parkinson [8]). It has been appreciated that the advance in our knowledge of the distribution of fungi in the rhizosphere has been restricted by technical problems of isolation. In an attempt

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to provide another approach to this problem a soil-box technique was devised (Parkinson [8]) and used in conjunction with a modified soil-plate technique (Warcup [18]).

These techniques were applied to rhizospheres of two crops of oats (variety S147), the crops being sampled at the following times by each method:

40	days	after	germination	(Young)
90	,,	,,	,,	(Early Maturity)
120	,,	,,	,,	(Maturity)
140	,,	,,	,,	(Late Maturity)
180	,,	,,	,,	(Senescence)

At each sampling fungi were isolated from the rhizosphere at three positions along the length of roots: root tip zone, crown zone, and intermediate zone (half-way between tip and crown).

(i) *Soil-plating technique*

Oats were grown in deep pots in a mixed garden loam and at each sampling a group of plants was carefully removed from the soil. Four plants from each group were selected and three main roots of each plant were used to yield the soil inocula for soil plates. Four Petri dishes were prepared for each zone of each root. The isolation medium was Czapek-Dox nutrient agar at pH 5.0 incubated at room temperature. The method of recording fungi was that suggested by Warcup [18], the four soil plates for each zone of each root being treated as a unit, a fungus being given a positive record if it occurred on one or more of the four plates. Soil samples at depths corresponding to the three root zones were taken some distance from the roots and were used as inocula for control plates.

(ii) *Soil-box technique*

Before beginning a series of isolations, six main roots were selected. Isolations were carried out by means of small tubes, half filled with plain water agar, and placed in position on the perspex face of the box. After seven days agar cores from these tubes were cut into segments, plated on Czapek-Dox agar pH 5.0 and incubated at room temperature (Parkinson [8]). Four plates were prepared for each isolation tube and a fungus was given a positive record if it appeared on one or more of the four plates. Using this technique isolations from the soil distant from the roots were also made at depths corresponding to those used for rhizosphere isolations, and at the same times as the rhizosphere isolations.

RESULTS

The data obtained from the application of the soil-plating and soil-box techniques as described above is presented in Tables 1 and 2, and in Figures 1, 2 and 3.

TABLE 1

Major groups of fungi isolated from three rhizosphere zones during the life of oat plants.
(Results expressed as percent of the total number of isolates at each isolation time)

Stage	Young			Early Maturity			Maturity			Late Maturity			Senescence		
Zone	Tip	Int.	Crown	Tip	Int.	Crown	Tip	Int.	Crown	Tip	Int.	Crown	Tip	Int.	Crown
	Isolated by the soil-box technique														
Mucoraceae	38.4	25.0	33.3	43.2	23.3	29.3	30.9	24.4	14.6	24.3	16.3	6.2	15.0	6.0	5.0
Mortierellaceae	15.4	44.4	25.0	10.2	31.1	21.9	14.3	26.8	21.9	18.9	25.5	12.4	12.5	10.0	10.0
Fusarium species	0.0	0.0	0.0	2.7	2.2	7.3	4.8	4.8	12.2	10.8	16.3	20.8	17.5	22.0	25.0
Sterile white mycelium	17.9	8.3	11.1	16.2	2.2	4.8	14.3	2.4	2.4	8.1	0.0	0.0	7.5	0.0	0.0
Sterile dark mycelium	0.0	0.0	0.0	0.0	2.2	2.4	4.8	2.4	7.3	2.7	2.3	8.3	7.5	8.0	6.6
Ascomycetes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	7.3	2.7	2.3	12.4	5.0	12.0	11.7
Sphaeropsidales	0.0	0.0	0.0	0.0	4.4	7.3	2.4	9.6	2.4	0.0	0.0	0.0	0.0	0.0	0.0
Dematiaceae	0.0	0.0	0.0	0.0	8.8	2.4	2.4	0.0	7.3	2.7	6.9	10.4	5.0	14.0	13.3
Other species	28.2	22.2	27.7	27.0	23.3	24.4	23.8	26.8	24.4	29.7	30.2	40.8	30.0	28.0	28.3
	Isolated by the soil-plating technique														
Mucoraceae	35.1	30.4	27.5	39.1	23.6	23.7	31.2	30.5	24.2	25.0	27.2	21.1	13.9	17.7	11.7
Mortierellaceae	6.6	11.3	13.3	8.7	11.6	7.7	7.6	12.4	7.1	4.6	8.7	5.7	2.5	6.2	2.9
Fusarium species	0.7	0.6	4.1	0.6	3.5	5.5	6.3	4.5	14.2	3.4	8.2	14.7	10.6	11.5	15.1
Sterile white mycelium	10.5	4.2	4.1	9.9	1.3	1.6	7.6	3.3	0.5	5.2	1.0	0.0	1.7	0.4	0.0
Sterile dark mycelium	0.7	0.6	0.7	0.0	0.4	3.2	1.9	0.0	0.0	2.8	1.5	5.3	5.0	3.6	4.1
Ascomycetes	2.6	2.9	1.4	3.7	6.2	2.2	3.8	3.3	3.8	5.8	4.5	4.9	6.7	5.8	7.1
Sphaeropsidales	0.0	4.2	1.4	0.0	4.4	3.2	0.6	3.3	4.9	3.4	3.0	1.6	8.0	3.2	5.0
Dematiaceae	10.5	10.1	8.3	9.0	13.6	8.8	9.5	9.6	9.8	11.0	10.2	14.7	17.3	15.5	19.6
Other species	31.5	35.7	38.6	28.5	38.4	43.6	31.2	36.1	35.1	38.4	35.3	32.1	33.9	35.4	34.3

TABLE 2

Major groups of fungi isolated from soil distant from roots at times similar to rhizosphere isolations, and at depths in the soil corresponding to the Tip, Intermediate and Crown zones of the rhizosphere isolations. (Results expressed as percent of total number of isolates at each isolation time)

Stage	Young			Early Maturity			Maturity			Late Maturity			Senescense		
Zone	Tip	Int.	Crown	Tip	Int.	Crown	Tip	Int.	Crown	Tip	Int.	Crown	Tip	Int.	Crown
	Isolated by the soil-box technique														
Mucoraceae	30.8	40.0	23.1	25.6	32.5	30.9	29.3	27.0	21.7	30.2	23.6	33.3	27.0	33.3	31.6
Mortierellaceae	25.6	25.6	30.8	30.8	25.0	23.8	26.8	37.8	26.1	30.2	26.3	26.2	32.4	25.0	21.1
Fusarium spp.	10.3	5.7	0.0	5.1	0.0	4.8	9.8	2.7	0.0	2.3	3.3	4.7	0.0	5.5	0.0
Sterile white mycelium	7.7	0.0	0.0	2.6	0.0	0.0	2.9	5.4	0.0	0.0	0.0	0.0	5.4	0.0	5.3
Sterile dark mycelium	0.0	0.0	5.1	0.0	0.0	0.0	2.9	0.0	0.0	9.2	0.0	0.0	0.0	2.8	0.0
Ascomycetes	0.0	5.7	2.6	10.2	0.0	7.2	4.9	0.0	6.5	0.0	6.6	4.7	2.7	0.0	5.3
Sphaeropsidales	0.0	0.0	0.0	2.6	2.5	0.0	2.9	0.0	4.4	0.0	6.6	2.4	0.0	0.0	0.0
Dematiaceae	0.0	0.0	5.1	0.0	5.0	0.0	2.9	0.0	2.2	0.0	0.0	4.7	5.4	0.0	0.0
Other species	25.6	22.8	33.4	25.6	35.0	33.3	19.5	27.0	39.2	27.6	36.8	23.8	27.0	33.3	36.8
	Isolated by the soil-plating technique														
Mucoraceae	24.4	21.8	23.2	16.2	22.7	23.4	23.3	25.5	24.2	19.9	24.8	26.2	23.8	21.5	26.4
Mortierellaceae	19.5	18.8	21.1	24.3	11.4	15.9	14.2	15.5	20.8	19.9	15.8	20.5	15.9	16.2	19.7
Fusarium species	4.9	3.9	3.2	6.1	8.1	6.4	0.0	5.5	6.6	6.9	4.9	1.9	4.5	7.5	4.4
Sterile white mycelium	0.0	1.9	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	2.9	0.0	0.0	0.0	3.3
Sterile dark mycelium	0.0	1.9	0.0	0.0	0.0	0.0	3.1	0.0	0.0	2.3	0.0	0.0	0.0	2.2	0.0
Ascomycetes	2.5	6.9	2.1	4.1	4.6	4.3	3.1	7.7	5.5	0.0	6.9	7.2	3.4	0.0	5.5
Sphaeropsidales	0.0	3.9	2.1	5.1	2.3	0.0	4.1	2.2	0.0	4.6	0.0	4.6	0.0	3.2	3.3
Dematiaceae	14.6	8.9	14.7	12.2	16.0	11.7	10.2	14.4	7.7	18.4	8.9	13.1	18.0	12.9	10.9
Other species	34.1	31.7	33.6	32.4	32.0	38.3	42.4	28.8	35.2	27.6	35.7	26.2	34.0	35.7	26.4

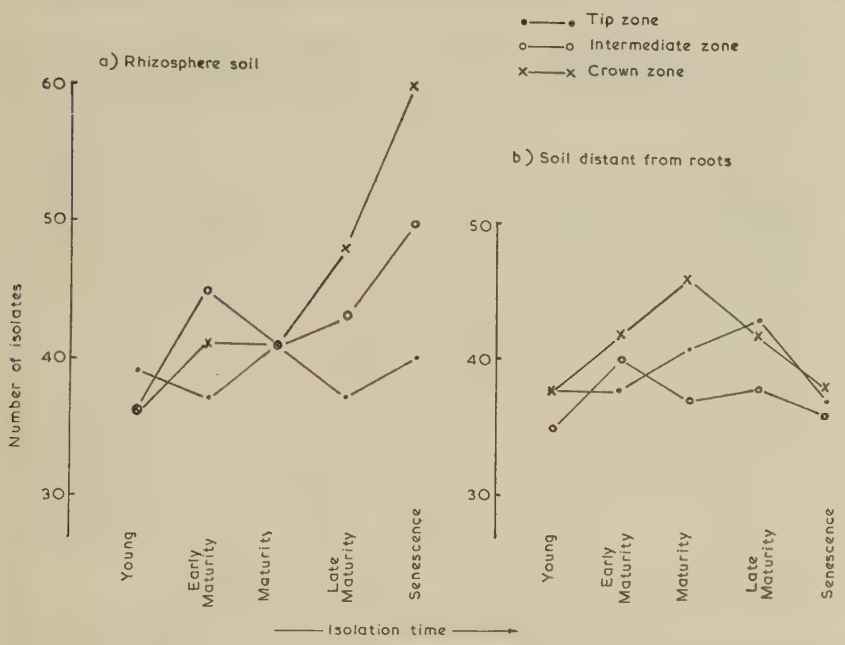

Fig. 1. Total number of isolates obtained from the three rhizosphere zones and from soil distant from roots at various stages of development of oat plants, using the soil-box technique.

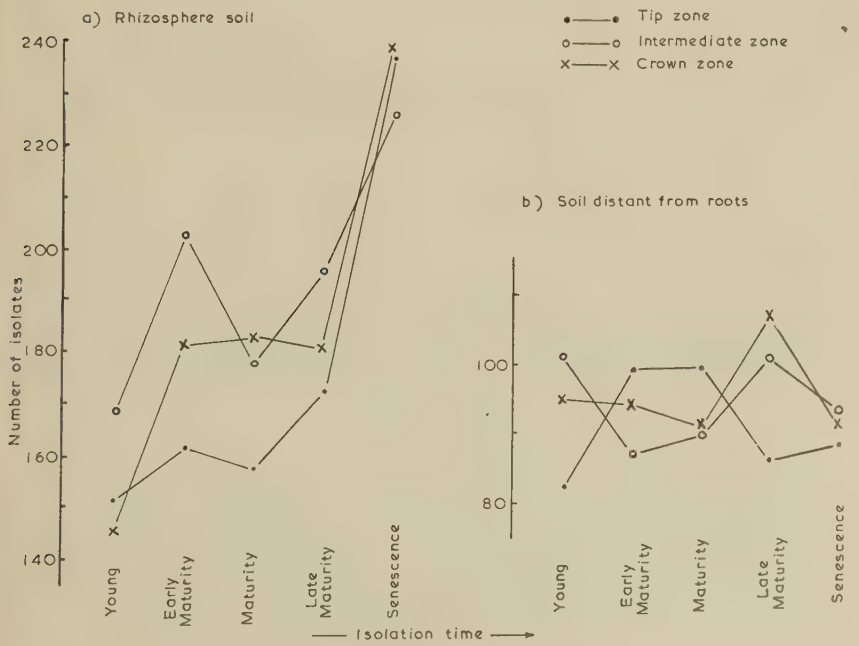

Fig. 2. Total number of isolates obtained from the three rhizosphere zones and from soil distant from roots at various stages of development of oat plants, using the soil-plating technique.

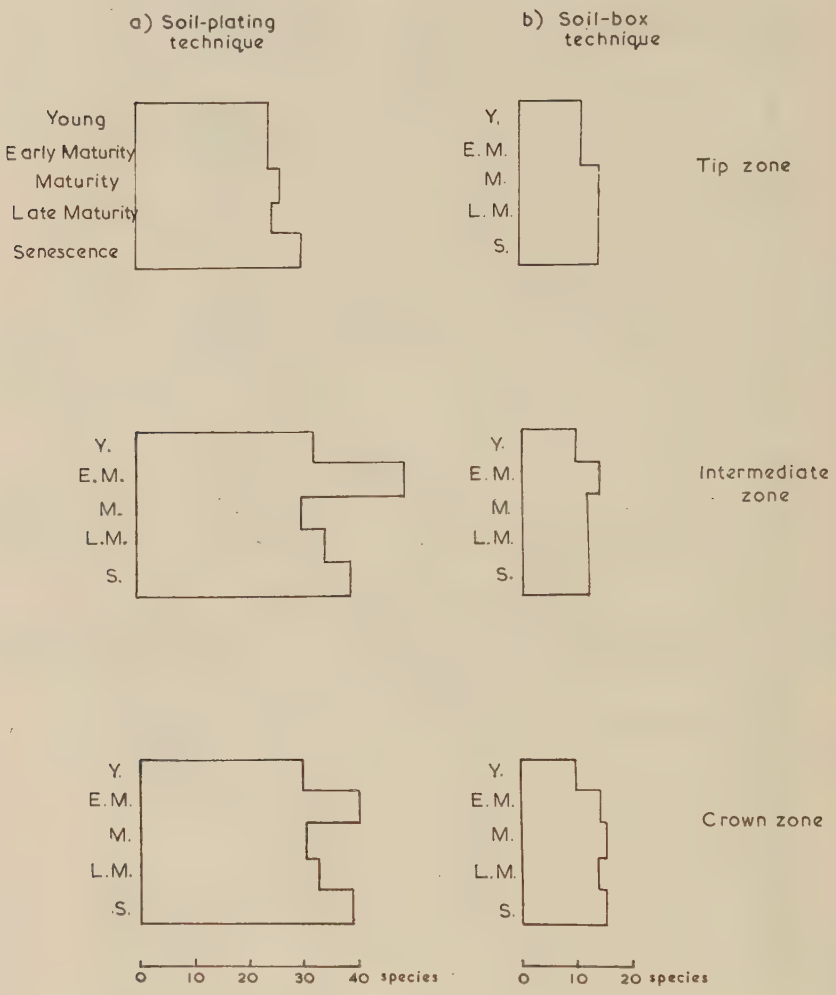

Fig. 3. Number of species isolated from each of the rhizosphere zones, at five stages of root development by the soil-plating and soil-box technique.

DISCUSSION

It is claimed that the techniques used in this investigation allow more detailed studies of the distribution of fungi in the rhizosphere than any hitherto in use. Since both are adaptations of standard methods for isolating soil fungi they possess disadvantages similar to those of the methods from which they were derived. Despite

intrinsic differences of method, the data which these techniques provide show marked similarities and essentially the same pattern of development of the rhizosphere microflora of oats is given by each.

In very general terms the succession of fungi in oat rhizospheres (Table 1) with increasing age of the roots commences with a population dominated by species of the Mucorales and by sterile hyaline mycelial forms. This is superceded by a population consisting mainly of species of Fusarium, of Dematiaceae, of sterile dark mycelial forms, and of Ascomycetes. The frequency of occurrence of *Trichoderma viride* and of species of Penicillium remains fairly constant throughout the life of the root system. Perhaps the most striking feature of this succession is the increase in incidence of *Fusarium culmorum* with the onset of senescence (Parkinson and Chesters [9]).

The zonal isolations provide data permitting conclusions to be drawn concerning successions of fungi in three root zones (Table 1). Thus, although each zone exhibits a similar development of its fungal population, the rate of development is not the same in each zone. Changes begin much earlier in the crown zone than in the tip zone; the Mucorales – sterile white mycelial community is maintained longest in the tip zone.

The data from soil distant from the roots (Table 2, Figures 1b and 2b) fail to show similar changes in the microflora at the three soil depths over the growing period of the oat plants. Hence the pattern of development of the rhizosphere microflora of oats appears to be peculiar to that microhabitat and to be a result of the changes in the soil brought about by the roots.

Stimulation of microbial development in the rhizosphere of higher plants has been ascribed to the exudation of organic and inorganic materials from roots, to the presence of dead root cells, and to the release of CO_2 by roots (Starkey [15], Harley [1]). With respect to exuded materials it has been demonstrated (Lundegårdh and Stenlid [6], Katznelson *et al.* [5], Rovira [10], Parkinson [7]) that substances such as sugars, amino acids and inorganic salts together with nucleotides and flavanones are present.

As the root tip region is one of great metabolic activity it is probable that the exudation of biologically reactive substances will be greatest in this region. The micro-organisms developing in

this zone will be selectively influenced by such exudates and might be considered as exudate indicator species. In the isolation data given for the root tip zone it is evident that, until maturity, the fungal population is dominated by species of the Mucorales (Table 1) The inability of species of this group to utilise complex carbohydrates and the fact that they exhibit rapid growth on sugars and inorganic sources of nitrogen may condition their presence in the growing areas and likewise their disappearance as regions of root tissues age and slough off cellulosic superficial tissues.

Changes in the fungal population of the crown zone appear at a much earlier stage in the life of the whole root system than those of the tip zone (Table 1). It is suggested that this is probably an effect of the presence of increased numbers of dead root cells superimposed on a decreased exudation of organic materials from this zone. Despite the recent increase of interest in the exudation of organic materials from roots, no data is available as yet on the actual sites of exudation, hence no quantitative data can be given regarding exudation from various root zones. Because of this it is only possible to postulate decreased exudation, but if the Mucorales may be considered as 'exudate indicator species' the decline in their frequency in the crown zone may lend support to this suggestion. The influence of dead root cells is demonstrated by the fact that with increasing age of the roots various active cellulose-decomposing fungi (e.g. *Chaetomium globosum, Stemphylium botryosum*) are isolated from the rhizosphere soil. A similar cellulose-attacking microflora begins to develop in the root tip zone at a time when the metabolic activities of that zone are declining and cells are dying. The increase in the frequency of occurrence of *Fusarium* species (particularly *Fusarium culmorum*) suggests the presence of newly dead plant tissues (Samuel and Greaney [12], Sadasivan [11]), and Waid [16] has demonstrated increased numbers of *Fusarium culmorum* with the degree of decomposition of ryegrass roots.

The isolations from the intermediate zone raise problems which, because of lack of precise knowledge of the physico-chemical features of the rhizosphere, are very difficult to resolve. The same features of fungal succession are exhibited as in the tip and crown zones, but species of Mortierella and of the Sphaeropsidales are isolated with greater frequency than from the other two zones, particularly during the earlier stages of root development.

Early work on the numerical fluctuations of rhizosphere-inhabiting micro-organisms (Starkey [15], Katznelson [3]) demonstrated that the largest numbers occurred when the higher plant is at the peak of its vegetative development. The present data allow a study of the number of fungal species occurring in each zone of the rhizosphere at each isolation (Figure 3). Both techniques show differences between the species numbers in each zone, but these differences are more marked in the 'soil plating' series, although in general terms, the same drift in numbers is reflected in the 'soil box' series. Referring to the former, it is evident that there has been an increase in the number of species recovered from the senescent portions of each zone. For the tip zone there is an increase from maturity to senescence; for both the intermediate and crown zones the general pattern is similar, but superimposed on this is a sudden increase at early maturity. If this increase is overlooked for the moment, the three zones differ mainly in the rather steeper increase in the number of species in the intermediate and crown zones as compared with the tip zone. This increase at senescence in each zone may be related to an increase in dead root materials at this time, but the increases at early maturity in the intermediate and crown zones, particularly the former, are difficult to explain at the moment. They may be due to an increase in root influences (Starkey [15]) through the effects of root exudates, combined with other factors as yet undetermined.

A comparison of the total number of species of fungi isolated from the three rhizosphere zones using the soil-plating and the soil-box techniques shows a larger number of species obtained by the former technique. This difference may either be due to the fact that the soil-box technique is selective for species in an active mycelial condition, or to the fact that competition for entry into, and survival in, the isolation tubes limits the number of species isolated. However, despite the numerical differences in species isolated, the two techniques do provide essentially similar pictures of the succession of dominant fungi in the three rhizosphere zones during the life of the oat plants.

It may be that the complementary use of two such techniques can provide valuable data on the form of the fungi in the rhizosphere (*i.e.* whether the fungi are in a sporing or in a vegetative phase). For example, in the isolation data provided it can be seen (Table 1)

that with the soil-box technique members of the Sphaeropsidales are isolated in the root-developmental stages of young, early maturity, and maturity, whereas by the soil-plate technique Sphaeropsidales are isolated at all stages of root development. If it could be ascertained that competition for entry into the isolation tubes is not a limiting factor it might be argued that between the young and mature root stages the species of the Sphaeropsidales are in an active mycelial state. During late maturity and senescence of the roots these species enter a sporing phase (and hence are isolated only by the soil-plating technique, which is not selective for active mycelium). Similar argument could be applied regarding the isolation data for Ascomycetes obtained by the two techniques. The abrupt appearance or disappearance of members of these groups in the soil-box isolates may lend support to this hypothesis, even if it is assumed that competition factors are limiting. Before definite statements can be made, isolation data must be accompanied by relevant physiological information on the sporing and vegetative growth of the species under consideration.

The difficulties of giving sound factual reasons for changes in distribution of fungi in rhizospheres emphasises a point so often made, that a more detailed approach to this problem is required, that it is necessary to study the distribution and physiology of restricted groups of fungi such as the Mucorales, sterile forms and Sphaeropsidales in rhizospheres, and to attempt a closer definition of the physical and chemical features of the rhizosphere, as has been attempted for pH by Seiberth [13].

SUMMARY

Tabulated information concerning isolations of fungi from growing roots of oat throughout the life of these roots by a method involving the removal of soil crumbs from roots (Warcup, soil plate [19]), and by a method presenting agar columns for infection *in situ* (Parkinson, soil box [8]) is provided, along with similar data concerning isolations from soil remote from root influences.

The results are discussed in relation to previous investigations of rhizospheres and it is shown that a succession of fungi in oat rhizosphere soil can be recognised. This is initiated by Mucorales and sterile hyaline mycelia and gives place to species of Fusarium, of Dematiaceae, of dark sterile mycelia and of Ascomycetes. Within root zones this succession moves at different time sequences. Attempts are made to explain these differences, but it is recognised that the limited knowledge which we possess of chemical

and physical changes of roots and of root exudates makes factual deductions almost impossible at the moment.

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ADSORPTIONSPROZESSE IM BODEN UND DIE WURZELERNÄHRUNG DER PFLANZEN *

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1. EINLEITUNG

Zur Zeit kann mann den Eintritt der Nährsalze in die Pflanzen durch das Wurzelsystem nicht zufriedenstellend erklären, ohne sich auf die Hypothese über den Adsorptionsaustausch der Ionen zwischen den Wurzelhärchen und dem sie umgebenden Film der Bodenlösung zu stützen. Als Austauschfonds der Pflanze dienen die Ionen der organischen Säuren, sowie die Wasserstoffionen und Bicarbonationen. Der Adsorptionscharakter der Aufnahme der Ionen durch Pflanzen gestattet es uns, die hohe Geschwindigkeit ihres Eintritts aus dem äußeren Medium zu erklären. Die mosaikartige Verteilung der positiven und negativen Ladungen des Protoplasmas, dank der verschiedenen Lage der isoelektrischen Punkte der ihr angehörenden Eiweiße, ermöglicht die gleichzeitige Adsorption auf verschiedenen Teilen des Protoplasmas, sowie der Kationen als auch Anionen aus dem Film der umgebenden Lösung.

Die Äquivalens des Austausches der Kationen zwischen dem Wurzelsystem der Pflanzen und der Außenlösung wurde wiederholtmalen überzeugend bewiesen. Dies geht aus den Arbeiten von Sabinin und Kolossov [21]. Jenny und Cowan [7] haben festgestellt, daß Sojabohnen-Sprößlinge, die Kalzium aus einem damit gesättigten Lehm aufnahmen, letzterem ein äquivalentes Quantum Wasserstoffionen abgaben. In dieser Hinsicht steht die Untersuchung von Blanc-Eckard [2] allein da, in der die Äquivalens des

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Austausches sowie auch der Austausch selber verneint werden. Dafür schlägt sie einen neuen Terminus vor: Wurzelsättigung mit Kationen. Den Anlaß zu dieser Annahme gab folgende Tatsache: die abgeschnittenen Wurzeln der Lupine absorbierten aus Kali- und Kalziumnitraten (gleicher Normalität) immer mehr Ca als K. Die Verdrängung von Kalium ging allerdings schwerer, als die des Kalziums vor sich.

Die obengenannte Verfasserin ist der Ansicht, daß diese Kationen von verschiedenen Gruppen festgehalten werden, die zur Wurzelhülle gehören. Dies verdient weiter untersucht zu werden.

Epstein [6], der Versuche mit abgeschnittenen Wurzeln der Gerste angestellt hatte, kam zu dem Schluß, daß etwa 23 Prozent des Volumens ihres Gewebes zur Zone der passiven Absorption des Kaliums und Kalziums gehören. Ihre Konzentration befindet sich im Gleichgewicht mit dem umgebenden Medium. Der übrige Teil des Volumens des Gewebes wird von demselben Verfasser zur Zone der aktiven Absorption gerechnet, in der folglich bereits ein Austausch stattfinden soll.

Es muß auch betont werden, daß die Fäden des Protoplasmas, die die Zellhülle durchdringen (Plasmodesmen), die bereits im Jahre 1883 vom russischen Botaniker Goroshankin [5] entdeckt wurden und in Jahre 1954 von Lambert [9] erneut sorgfältig untersucht wurden, an der aktiven Absorption, d.h. am Austausch von Ionen mit der Außenlösung teilnehmen können *. Wie schon Gedroiz [4] festgestellt hat, gibt es in jedem salzfreien Boden dutzend, ja hundert Mal mehr Kationen im austausch- adsorbierten Zustand, als im wasserlöslichen. Auf dieselbe Weise werden auch im Boden die Kationen verteilt, die mit den Mineraldüngern eingebracht werden, oder bei der Mineralisierung der organischen Dünger auftreten. In den letzten Jahren wurde auch die Austauschabsorption von Anionen durch den Boden bestätigt, bisher zwar nur am Beispiel der Phosphorsäure. Das bedeutet aber, daß die Ernährung der Pflanzen mit allen Kationen und einigen Anionen (außer den Nitrationen) unter natürlichen Bedingungen mehr von ihrer Anwesenheit im adsorbierten Zustand, als im wasserlöslichen abhängt.

Im Zusammenhang damit ist das Studium der Bedingungen, die

* Wertvolle Hinweise gaben auch die Forschungen von Strugger [25].

die Pflanzenaufnehmbarkeit der vom Boden adsorbierten Ionen erklären sehr wichtig, um so mehr, als im Verlaufe einiger Jahrzehnte die Meinung in der Agrochemie vorherrschte, die Pflanze selbst sei unfähig, die vom Boden adsorbierten Kationen aufzunehmen so lange sie nicht infolge von Austauschreaktionen, die unabhängig von der Lebenstätigkeit der Pflanze vorsichgehen, in Lösung überführt sind.

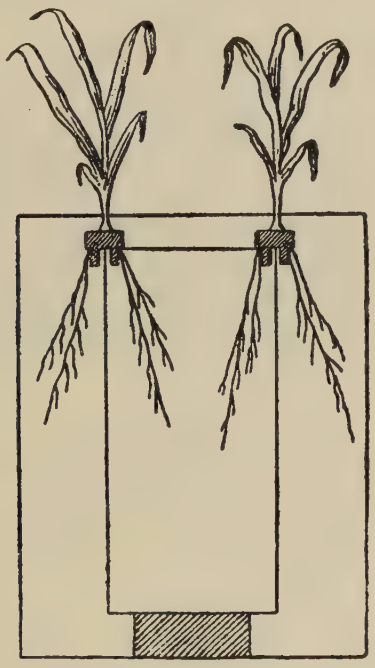

Abb. 1. Schematische Darstellung der methode der isolierten Pflanzenernährung.

Diese Meinung kam auf Grund der Versuche an Pflanzen zustande deren Wurzelsystem in zwei Teile, die in getrennten Gefäßen untergebracht wurden, aufgegliedert war. In dem einen befand sich eine Nährsalzlösung (ohne Kali), in dem anderen kaligesättigter Permutit. Bei diesen Versuchsbedingungen haben Tschirikow [26 27], Smirnow [24] und Bobko [3] zu dem sich im Permutit befindenden Teil des Wurzelsystems der Gerste weder Kalziumspuren, noch Mikroelemente hinzugefügt, und dies hat unserer Meinung nach

den anormalen Zustand und die schwache Absorptionsfähigkeit der entstehenden Wurzelverzweigungen bedingt.

Bald darauf wurde bekannt, daß gerade Kalzium, Eisen, Bor und Mangan sich in der Pflanze nicht von oben nach unten fortbewegen und aus dem einen Teil der Wurzel nicht in den anderen übergehen können. Wir haben diese Tatsache an den Wurzeln von Winterroggen, Hafer und Erbsen unter Verwendung von radioaktiven Calzium (Ca^{45}) gezeigt (Peterburgsky [18]).

2. AUFNEHMBARKEIT ADSORBIERTER IONEN

Unser Versuch (Peterburgsky [11]) mit Gerste mit getrenntem Wurzelsystem – im äußeren Gefäß Hellriegel-Mischung ohne Kali, im inneren Gefäß Permutit, gesättigt mit Kali – hat in der Tat bestätigt, daß eine genügende Aufnahme des Kaliums aus dem Adsorbenz durch die Pflanze durchaus möglich ist, falls die Wurzeln über die gewöhnliche Menge von Bor, Mangan und eine geringe Menge Calzium ((1/24 von der Hellriegel-Norm) verfügen. Die Versuchsergebnisse sind in der Tabelle 1 angeführt.

TABELLE 1

Kaliaufnahme durch Gerste (in me pro Gefäß) aus K-Permutit			
Versuchsvarianten		K-Gehalt in den oberirdischen Masse	Anmerkungen
äußeres Gefäß	inneres Gefäß		
Hellriegel-Mischung ohne Kali	K-Permutit	0,20	ohne Ca, B, Mn im inneren Gefäß
Hellriegel-Mischung ohne Kali und in beiden Gefäßen	K-Permutit	0,86	
Hellriegel-Mischung ohne Kali	K-Permutit	3,08	mit Ca, B, Mn im inneren Gefäß
Hellriegel-Mischung ohne Kali und in beiden Gefäßen	K-Permutit	7,72	

Der Kaligehalt in den Sprößlingen zu Beginn ihres Wachstums auf der Nährlösung ist hierbei abgezogen. Bei Zugabe von Ca, B und Mn wurde außerdem auch noch die Kalimenge abgezogen, die durch Hinzufügen von Kalzium aus Permutit verdrängt, und dabei von der Pflanze als gelöstes Kali aufgenommen wurde. Des weiteren wurde eine bedeutende Anzahl von Versuchen (Peterburgsky [12])

mit Sprößlingen verschiedener Kulturen durchgeführt, die vorher auf Nährlösungen mit einem unbedeutenden Kaligehalt herangezogen waren und danach auf Suspensionen von mit Kali gesättigten Adsorbenten und Böden verpflanzt wurden. Den Suspensionen wurden keinerlei andere Nährsalze beigefügt, da es sich um kurzfristige Versuche handelte. Die erzielten Ergebnisse haben die Pflanzenaufnehmbarkeit des Kaliums aus den Adsorbenten vollauf bewiesen. Hierbei fehlten Elektrolyten, welche, unabhängig von der Lebenstätigkeit der Pflanzen, adsorbiertes Kali aus dem Boden austauschen könnten. Zum Vergleich wurden in die Versuche Varianten eingeschaltet, bei denen die Wurzeln für dieselbe Zeitdauer in KCl-Lösungen getaucht wurden, die dieselbe Menge an K enthielten wie die Suspensionen.

In Tabelle 2 sind Ergebnisse eines typischen Experimentes dieser Art angeführt, bei dem die Gerstensprößlinge in Lösungen von KCl oder in Suspensionen von Askanitton aus Grusiën (Montmorillonit-Typ) gehalten wurden. In beiden Fällen betrug der K-Gehalt der Pflanze 4 me pro Gefäß.

TABELLE 2

Kaliaufnahme durch Gerste (in % von der eingebrachten Kaligabe)			
Versuchsdauer (Stunden)	Aufgenommenes K aus		Anmerkungen
	KCl	K-Askanit	
48	21,2	13,2	vorher wurden die Keimlinge im Verlauf von zwei Wochen auf Nährlösung ohne Kali gehalten
144	43,2	28,0	
288	40,5	40,5	

Die gleichen Ergebnisse sind auf Sandkulturen durch Einbringen von Adsorbenten erzielt worden, sowie auf Böden, die eine verschiedene Kalisättigung aufwiesen und eine Zugabe von anderen Nährstoffen nicht erhielten. Betrachten wir einen dieser Versuche, der auf zwei Bodentypen durchgeführt wurde; auf lehmiger, ausgelaugter Schwarzerde des Kiëwer Gebiets und auf Rasenpodsolboden aus dem Moskauer Gebiet (Tabelle 3).

Die angeführten Zahlen zeugen davon, daß mit Ausnahme eines Falles die Erhöhung des Sättigungsgrads des Bodens mit Kali gleichzeitig auch dessen Pflanzenaufnehmbarkeit gesteigert hat. Da die Kalidosen in allen Varianten gleich waren, stellte es sich

heraus, daß er aus dem podsoligen Boden weniger aufnehmbar war, als aus dem Tschernosjom.

TABELLE 3

Einwirkung der Sättigung (in % von der Sorptionskapazität) des Bodens mit Kali, auf dessen Aufnahme (in mg auf 1 g trockener Pflanzenmasse) durch Sonnenblumen

Schwarzerde				
K-Sättigungsgrad	4,6	6,1	9,1	13,9
Aufgenommenes-K	21,7	27,1	39,5	41,9
Rasenpodsol				
K-Sättigungsgrad	7,9	16,3	22,8	34,9
Aufgenommenes-K	10,1	14,7	18,1	5,9

3. DIREKTER EINFLUSS DER WURZEL AUF ADSORBENTEN.

Über die Bedeutung der Hydrolyse des Adsorbenten, die unabhängig von der Einwirkung der Wurzelausscheidungen verläuft, wurden zahlreiche Versuche angestellt, deren Gesammtschema von Peterburgsky [13] dargestellt wurde (Abb. 2). Tabelle 4 zeigt Ergebnisses eine Versuches mit Sonnenblumensprößlingen auf Suspensionen der zwei oben beschriebenen Böden.

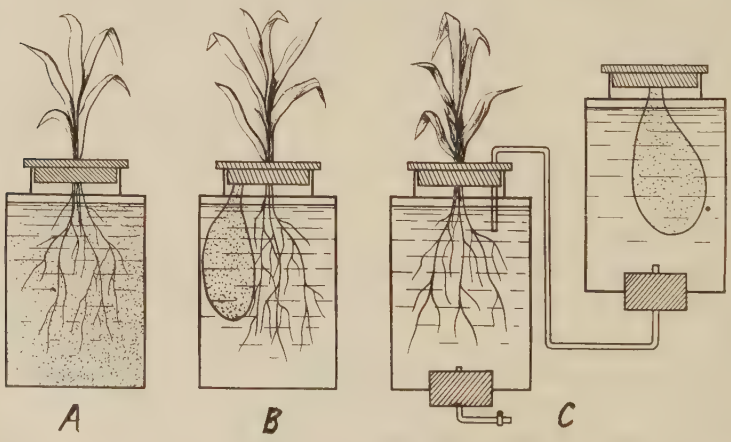

Abb. 2. Allgemeines Schema des Studiums der Ionen aufnahme der Pflanzen aus dem Adsorbenten:

A — Suspension in unmittelbarem kontakt mit Wurzeln.
B — Suspension ohne kontakt mit den Wurzeln (Kollodiummembrane).
C — Hydrolyse des Adsorbenten.

TABELLE 4

Bedeutung der Hydrolyse, der Einwirkung von Wurzelausscheidungen und des unmittelbaren Wurzelkontaktes auf die Aufnehmbarkeit des Kaliums aus den Adsorbenten durch Sonnenblumen

Quelle des Austausch -K	Aufgenommenes K (in mg auf 1 g Trockenmasse)		
	A	B	C
K-Tschernosjom	3,39	1,15	0,01
K-Podsol	3,54	1,37	0,28

Die Versuchsergebnisse liefern einen endgültigen Beweis dafür daß die Kalimenge, die von der Sonnenblume aus der festen Phase aufgenommen wird, nicht von der Hydrolyse des Adsorbenten, sondern von der Einwirkung des Wurzelsystems der Pflanzen auf denselben bestimmt wird. Diese Einwirkung macht sich beim unmittelbaren Kontakt der Wurzeln mit den Bodenteilchen am meisten bemerkbar.

Was den Mechanismus der Aufnahme der vom Boden adsorbierten K-Ionen durch die Wurzeln betrifft, so kann man die Hypothese von Jenny und Overstreet [8] bisher noch nicht als bewiesen betrachten – es handele sich um ein „Überspringen", d.h. um einen direkten Kationenaustausch zwischen den Myzellen des Adsorbenten und der Oberfläche des Wurzelhärchens, angeblich ohne die Lösung zu passieren. Obwohl die Versuchsergebnisse dieser Wissenschaftler von außerordentlichem Interesse sind, kan man die Tatsache nicht außer Acht lassen, daß sie mit Tonsuspensionen gearbeitet haben, in welche die Gerstenwurzeln getaucht wurden, und folglich eine Wechselwirkung durch die Lösung nicht vermieden werden konnte *. Der in den Arbeiten von Möller [10], Antipow-Karatajew [1], Scharow [22], Wiklander [28] und Sengupta [23] bewiesene gegenseitige Austausch von adsorbierten Kationen zwischen den Myzellen verschiedener Adsorbenten (darunter auch Böden) ging in einem feuchten Medium vor sich. Unserer Ansicht nach geschah dies infolge der Übertragung von Kationen durch die Anionen OH^- und HCO_3^-, die in Wasser und in Wassersuspensionen vorhanden sind. In Abb. 3 bringen wir das Schema einer analogen Beteiligung dieser Anionen in der Wechsel-

* Analogische Schlußfolgerungen machte in seinen Arbeiten auch Ratner [20].

wirkung zwischen dem Wurzelhärchen und den kolloidalen Bodenteilchen.

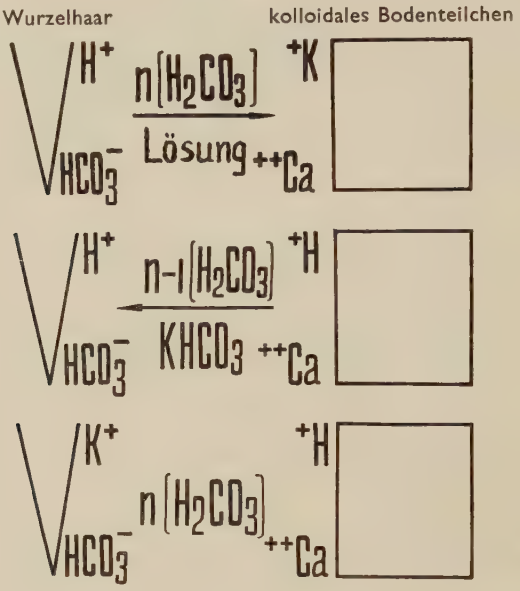

Abb. 3. Schema der Wechselwirkung zwischen Pflanze und Boden durch die Lösung.

4. MÖGLICHKEIT GUTEN WACHSTUMS AUF SUBSTRAT MIT ADSORBIERTEN IONEN

Bei Anwendung synthetischer Kationite und Anionite haben wir auf Sandkulturen (Peterburgsky [14 15 16]) mit Sonnenblumen, Weizen (Abb. 4), Hafer (Abb. 5), Zuckerrüben, Tomaten u.a. Pflanzen ihr zufriedenstellendes Wachstum in einem Medium gezeigt, das keine lösbaren Salze enthält. In den Sand wurden lediglich Adsorbenten eingebracht, die mit den entsprechenden Kationen und Anionen gesättigt waren. Erst sollen die Ergebnisse des Versuches mit Weizen betrachtet werden (Tab. 5), wobei die Entwicklung des letzteren auf einer Hellriegel-Nährlösung und auf Mischungen von adsorbierten Ionen verglichen wurde. Drei Wochen nach dem Auflaufen wurde ein Teil der Gefäße mit Wasser durchgespült. Auf den Gefäßen mit der Nährsalzlösung wurde das

TABELLE 5

Versuchsvarianten	Ertrag und Nährstoffgehalt des Weizens							
	Gewicht (i.g. pro Gefäß)		Gehalt an Nährstoffen in der Ernte in me					
	Korn	Stroh	N	K	Ca	Mg	SO_4	P_2O_5
Nährsalzlösung ohne Durchspülung . . .	7,2	19,1	18,3	12,1	7,2	2,4	6,0	6,9
Nährsalzlösung mit Durchspülung . . .	0,5	5,5	—	—	—	—	—	—
Gemisch von adsorbierten Ionen ohne Durchspülung . . .	3,7	9,8	8,2	4,8	2,3	1,4	3,3	4,6
Gemisch von adsorbierten Ionen mit Durchspülung . . .	3,0	9,0	—	—	—	—	—	—

Abb. 4. Zustand des Weizens:

2. Normale Nährsalzlösung nach Hellriegel ohne Durchspülung mit destiliertem Wasser.
4. Normale Nährsalzlösung nach Durchpülung mit Wasser;
6. Gemisch von adsorbierten Ionen ohne Durchspülung;
8. Gemisch von adsorbierten Ionen nach Durch spülung mit Wasser;
10. 0,5 normale Nährlösung nach Hellriegel;
12. 0,5 normales Gemisch von adsorbierten Ionen.

Abb. 5. Nährstoffdosen im Versuch mit Hafer. Verabreicht als Nährsalze: 3) — 0,5 Norm, 1) — 1 Norm, 5) — 2 Normen; Als adsorbierte Ionen: 9) — 0,5 Norm, 7) — 1 Norm, 11) — 2 Normen, 13) — 3 Normen, 15) — 4 Normen.

Wachstum hierdurch stark beeinträchtigt; dagegen blieb die Weiterentwicklung der Pflanzen auf der Mischung mit adsorbierten Ionen, nahezu unbeeinflußt.

Das Titrieren der Salsauszüge aus dem Sand nach dem Abernten des Weizens wies einen Anhäufung von H-Ionen in den Gefäßen mit Adsorbenten nach, die größer ist, als in den mit Salzlösungen. Das zeugt davon, daß der Austausch und die Absorption der Basen durch Pflanzen auf Kosten der von den Wurzeln ausgeatmeten Kohlensäure vor sich ging.

In Bodenkulturen, wo der Kationen- und Anionenaustausch dank einer mehr oder minder ausgeprägten Konzentration der Elektrolyten intensiver verläuft, stehen die Adsorbentenmischungen den Salzmischungen in nichts nach, mehr noch, sie waren ihnen gewöhnlich noch überlegen. Dies ist durch das Fehlen der physiologischen Azidität bei der Ernährung der Pflanzen mit Kationen die aus dem Medium adsorbiert wurden zu erklären. Aus der Viel-

zahl derartiger Versuche wollen wir nur auf die Ergebnisse des einen hinweisen, der auf sauren Rasenpodsolboden mit Klee durchgeführt wurde (Tabelle 6).

TABELLE 6

Ertrag des Rotklees und sein Nährstoffgehalt					
Versuchsvarianten	Gewicht in g	Gehalt an Nährstoffen (in me pro Gefäß)			
		N	K	Ca	P_2O_5
Ohne Dünger u. Kalk	12,2	12,7	9,5	11,0	2,7
Kalkung auf 25% der hydrolytischen Azidität + PK (Salz)	15,5	13,9	12,8	13,1	3,5
Kalkung auf 25% der hydrolytischen Azidität + PK (Adsorbenten). . . .	22,1	17,4	16,1	21,9	4,7

An den Sandkulturen mit Radieschen und den Bodenkulturen mit Hafer wurde die Pfanzenaufnehmbarkeit der Nitrat-, Sulfat- und Phosphatanionen bewiesen, wenn sie in adsorbierter Form eingebracht werden (Peterburgsky [19]). Für $H_2PO_4^-$ (Monophosphation) wurde dies auch unter Verwendung von radioaktiven Phosphor (P^{32}) gezeigt. Das Impfen des Sandes mit Rhizosphäre-Mikroorganismen des Hafers hat dessen Aufnahmefähigkeit von Nährstoffen weder aus den Lösungen noch aus den Adsorbenten intensiviert. In sterilen Verhältnissen besaßen die Haferpflänzchen die gleiche Aufnahmefähigkeit. Deshalb kann man zum Schluß darauf hinweisen, daß auch die Pflanze selbst in der Lage ist, dank ihrer Wurzelausscheidungen, die vom Bodensorptionskomplex adsorbierten Ionen loszulösen und sie aufzunehmen (Peterburgsky [17]).

Die Prozesse der Austauschverdrängung der vom Boden adsorbierten Ionen in die Lösung, die unabhängig von der Lebenstätigkeit der Pflanze vorsichgehen, bilden natürlich ebenfalls einen wichtigen Faktor der Wurzelernährung.

5. ZUSAMMENFASSUNG

1. Eine große Rolle bei der Ernährung der Pflanzen spielen die von Bodenkolloiden adsorbierten Kationen und möglicherweise auch die Anionen, ins besondere die Phosphationen.

2. Die vom Boden adsorbierten Ionen können im feuchten Medium von einem Kolloidteilchen auf ein anderes migrieren. Analoge Prozesse können

auch zwischen Bodenteilchen und Wurzelhärchen durch den sie trennenden Lösungsfilm vorsichgehen.

3. Die durchgeführten Versuche haben gezeigt, daß die Pflanzen sich ernähren und einen befriedigenden Ertrag liefern können, wenn sie auf einem Substrat gezogen werden, in welchem keine Salze, sondern nur adsorbierte Ionen vorhanden sind.

4. Die Pflanzenaufnehnbarkeit der vom Boden adsorbierten Ionen wurde unter Verwendung verschiedener Methoden bewiesen und zwar: der Sämlingsmethode, der Methode der getrennten Wurzelmasse und der Mischkulturen, unter Ausnutzung verschiedener Adsorbenten (künstliche Zeolithe, synthetische Harze, Tone und Böden), und auch durch Anwendung radioaktiver Isotope.

5. Die wichtigste Voraussetzung zur Ausnutzung der adsorbierten Ionen durch die Pflanzen, ist der enge Kontakt zwischen dem Wurzelsystem und der festen Phase des Bodens.

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ÉTUDE DE LA DÉCOMPOSITION DE LA MATIÈRE ORGANIQUE DANS LE SOL AU MOYEN DE CARBONE RADIOACTIF *

III. DÉCOMPOSITION DES SUBSTANCES SOLUBLES DIALYSABLES, DES PROTÉINES ET DES HÉMICELLULOSES

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I. INTRODUCTION

Faisant suite à l'étude de la décomposition du glucose C^{14} dans le sol [2 3], on a entrepris celle de la répartition de la radioactivité dans les diverses fractions humiques obtenues aux dépens de substrats radioactifs d'origine végétale. On donne ci-après les résultats obtenus à partir des substances solubles dialysables et non dialysables (protéines) du ray-grass ainsi que des hémicelluloses. Cette étude a permis d'établir une analogie entre la décomposition de ces divers substrats et celle du glucose.

II. MÉTHODES EXPÉRIMENTALES

A. *Substances* C^{14} *solubles dialysables du ray-grass*

La préparation de cette fraction ainsi que son analyse chromatographique est décrite dans une précédente publication [1].

B. *Substances* C^{14} *solubles non dialysables du ray-grass* (*proteines* C^{14})

On a obtenu des protéines végétales radioactives en opérant de la façon suivante:

Un gramme de feuilles adultes de ray-grass, rendues radioactives par photosynthèse aux dépens de 1 mc $BaC^{14}O_3$ (v. Bibl. [1]) sont broyées au pillon avec 5 g de sable fin et 10 ml tampon phosphate 0,1 *M* pH = 7,0 à 4° C. Cette masse est ensuite filtrée au travers d'un

* Travail effectué sous les auspices de l'Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (I.R.S.I.A.)

filtre Jena IG 3, préalablement recouvert de celite analytique. On lave avec 15 ml de tampon phosphate 0,1 *M* pH = 7,0; On introduit le filtrat dans un sac de cellophane et on dialyse pendant 48 heures, dans un litre de tampon à 0,005 *M* pH = 7,0 à 4° C, en renouvelant le tampon toutes les 8 heures. La radioactivité de la fraction est très approximativement celle indiquée dans un autre travail [1].

Après hydrolyse, on distingue par analyse chromatographique dans cette fraction plus de 10 acides aminés. On a trouvé l'acide aspartique (40,4%), l'acide glutamique (12%), la sérine (7%), la glycine (7,6%), l'alanine (4,5%), la tyrosine (3,4%), la valine (2%), la phénylalanine (7,2%), la leucine (7%), la proline (0,9%) et d'autres substances (7%).

C. *Hémicellulose* C^{14}

La préparation et l'analyse chromatographique de cette fraction a été décrite ailleurs [1].

D. *Decomposition des substances organiques dans le sol et fractionnement des complexes humiques*

On opère sur une quantité de substrat voisin de 4 mg de carbone que l'on introduit dans une quantité de sol correspondant à 4 g de poids sec, l'humidité restant maintenue à 45% et la température à 25° C.

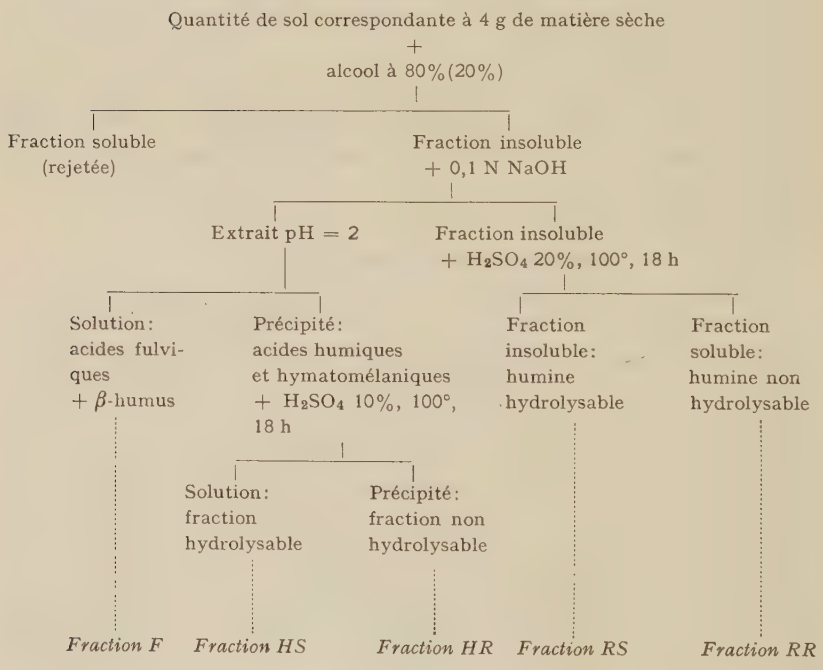
Après 30 jours on traite le sol suivant le schéma suivant qui est plus simple que celui suivi précédemment [2].

On extrait comme décrit précédemment la fraction soluble à l'aide de solutions alcooliques bouillantes à 80 et 20%. Après cette extraction on met le sol en suspension dans 15 ml NaOH 0,1 *N*. On extrait les acides humiques à 100° C sous atmosphère d'azote pendant 60 minutes. Par centrifugation à 20.000 t/m on sépare le sol de la solution surnageante; on lave le sol avec 10 ml NaOH 0,1 *N* et on centrifuge. On réunit les extraits alcalins qu'on acidifie avec 2 ml H_2SO_4 20% et on centrifuge à 3000 t/m. Le surnageant est constitué des acides fulviques et du β-humus. Cette fraction est représentée par la lettre F. On resolubilise, par addition de 5 ml NaOH 0,1 *N*, le culot de centrifugation composé des acides humiques et des acides hymatomélaniques; on rince deux fois avec 2,5 ml 0,1 *N* NaOH et on porte ces solutions alcalines dans un tube à hydrolyse, pourvu d'un bulbe de 40 ml de capacité et analogue à

celui utilisé dans l'étude de la décomposition du glucose radioactif [3]. On ajoute 10 ml H_2SO_4 20% ; on porte à 100° C pendant 18 heures, l'hydrolyse se faisant sous atmosphère d'azote. Après hydrolyse on sépare, par centrifugation, le surnageant représentant la fraction hydrolysable HS, du résidu insoluble qui représente la fraction HR ou fraction non hydrolysable des acides humiques et hymatomélaniques. La mesure de la radioactivité des fractions F, HR et HS se détermine sur la moitié de l'échantillon, c'est à dire sur une fraction qui correspond à 2 g de sol sec. L'humine hydrolysable (fraction RS) et l'humine non hydrolysable (fraction RR) se déterminent sur l'équivalent de 0,5 g de sol sec après hydrolyse en tube scellé avec 5 ml H_2SO_4 20% à 100° C pendant 18 heures. Le tableau 1 donne le schéma de ce fractionnement.

TABLEAU 1

Schéma de fractionnement de la matière organique du sol



La mesure de la radioactivité ne pouvant sans erreur se déterminer sur l'échantillon tel quel, on oxyde quantitativement en

anhydride carbonique chacune des fractions humiques par chauffage avec le mélange de Van Slyke *. On recueille le $C^{14}O_2$ sous forme de $BaC^{14}O_3$ dont on mesure la radioactivité [4 5 6].

Voici les détails de ces manipulations: On introduit dans un ballon de 100 ml la solution des fractions humiques, on y ajoute une goutte d'alcool octylique comme anti-mousse et on en réduit le volume à 0,5–1 ml par évaporation sous vide dans un évaporateur rotatif (Aloë, Rinco Trust Co., Greenville, USA). On raccorde le ballon au dispositif schématisé fig. 1 en

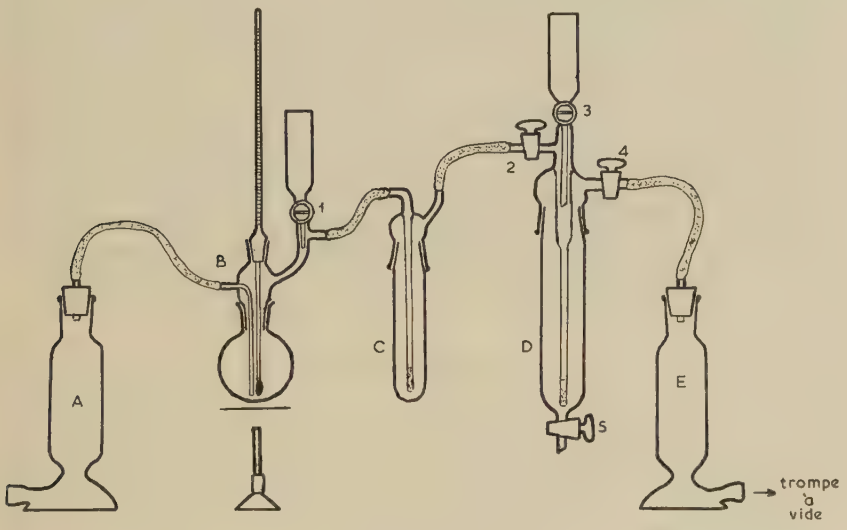


Fig. 1. Appareil de Van Slyke.
A, E: Trappe à chaux sodée
B: Générateur avec thermomètre
C: Trappe à bichromate
D: Tube à absorption

ayant soin d'enduire de H_3PO_4 syrupeux le joint et le robinet 1. Entre le générateur (B) et le tube à absorption (D) on place une trappe (C) de bichromate (solution à 2% dans H_2SO_4 10 *N*) pour eliminer les oxydes d'azote et de souffre qui éventuellement pourraient se former par chauffage. Pendant 5 minutes on aspire au au travers du dispositif de l'air qui est débarrassé de CO_2 par passage dans le tube de chaux sodée (A). On ferme ensuite le robinet 2, on ouvre 3 et on verse dans le tube à absorption 60 ml. NaOH *N* suivi de 60 ml d'eau distillée. On ferme 3, on ouvre 2 et on maintient dans l'appareil une légère dépression telle qu'il y ait un barbotage d'air dans la

* CrO_3 25 g, KIO_3 5 g, H_3PO_4 85% 167 ml, H_2SO_4 conc. 333 ml. On chauffe jusqu'à 150° et laisse refroidir avec un verre de montre sur l'orifice du ballon.

trappe (D) (tube barboteur Corning 395 33C); on ouvre le robinet 1, on introduit 30 ml du mélange Van Slyke. On ferme le robinet 1, on chauffe le ballon jusqu'à 140° C. On maintient la température pendant 15 minutes entre 130 et 150° C. Tout en arrêtant le chauffage, on maintient cependant encore pendant 5 minutes le barbotage. Pour verser dans un erlenmeyer la solution alcaline du tube (D), on ferme le robinet 2, on déconnecte (E) de la trompe à eau, on maintient ouvert le robinet 4 et on ouvre le robinet 5. On rince le tube à absorption (D) avec 200 ml d'eau distillée qu'on ajoute à la solution primitive. On ajoute 5 ml NH_4NO_3 (350 g/l) et 5 ml $BaCl_2$ (300 g/l) et on attend une nuit que le $BaC^{14}O_3$ se dépose au fond de l'erlenmeyer. La majeure partie du liquide est ensuite éliminée par aspiration, tandis que la suspension de $BaC^{14}O_3$, préalablement chauffée au bain-marie, est reprise quantitativement sur un filtre Iena 63aIG 4 maintenu sous dépression. On lave le $BaC^{14}O_3$ à l'eau bouillante et à l'alcool éthylique. On sèche à 120° C.

Le poids du $BaC^{14}O_3$ moins le poids du témoin (de l'ordre de 5 mg) multiplié par 0,06085 donne le poids du carbone contenu dans l'échantillon. On étend ensuite de 1 à 10 mg de $BaC^{14}O_3$ sur une plaquette d'aluminium selon la méthode décrite par Calvin et on mesure la radioactivité de l'échantillon à l'aide d'un Flow Counter "Nuclear" (1 coup = 3,4 désintégrations). La radioactivité réelle est déterminée en tenant compte des corrections de coïncidence et de self-absorption [5].

III. RÉSULTATS

Le tableau 2 donne la répartition de la radioactivité dans les diverses fractions humiques obtenues après décomposition dans le sol des substances solubles dialysables et non dialysables (protéines) ainsi que des hémicelluloses.

La radioactivité résiduelle des fractions humiques est pour chacun des substrats voisine de 20% de la valeur initiale. Dans tous les cas, elle est plus élevée que pour le glucose. Comme cela avait déjà été constaté auparavant [1], la répartition de la radioactivité entre les différentes fractions humiques ne varie guère. En outre, ce sont les fractions F, HS et RS qui sont toujours les plus actives et totalisent, comme dans le cas du glucose, près de 80% de la radioactivité résiduelle. La radioactivité dans la fraction HR est faible, ce qui amène à dire que l'origine de cette fraction ne se trouve pas dans une décomposition des protéines ou des hydrates de carbone solubles. L'étude de la décomposition de la cellulose et de la lignine déterminera sans doute le substrat donnant naissance à la fraction HR. Eu égard aux fortes radioactivités rencontrées dans les fractions F, HS, RS il se confirme que ces fractions tirent leur origine de la

TABLEAU 2

Radioactivité des fractions humiques du sol obtenues aux dépens des substances C^{14} solubles dialysables, des protéines C^{14} et des hemicelluloses C^{14}. La décomposition du glucose C^{14} est donnée à titre de comparaison.

Substrat.	Subst. C^{14} solubles dialysables	Protéines C^{14}	Hemicelluloses C^{14}	Glucose C^{14}
Radioactivité * initiale	18.224	1.862	1.405	6.100
résiduelle **	3.924 (21,6%)	460 (24,6%)	282 (20,1%)	690 (11,3%)
Fractions humiques †	Répartition de la radioactivité ††			
F	33,97	18,04	22,35	20,28
HS	23,16	36,18	31,88	29,80
HR	4,63	7,52	9,91	4,75
RS	25,09	24,48	23,25	31,26
RR	13,12	13,74	12,58	13,88

* Radioactivité exprimée en mille coups par minute.

** Pourcentage par rapport à la radioactivité initiale de l'ensemble des fractions humique abstraction faite des substances solubles dans l'alcool à 80 et 20%.

† Signification des abréviations: voir méthodes expérimentales.

†† Répartition de la radioactivité exprimée en pourcentage par rapport à la radioactivité résiduelle du sol.

décomposition microbienne des hydrates de carbone et des protéines.

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ÉTUDE DE LA DÉCOMPOSITION DE LA MATIÈRE ORGANIQUE DANS LE SOL AU MOYEN DE CARBONE RADIOACTIF *

IV. DÉCOMPOSITION DES PIGMENTS FOLIAIRES

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I. INTRODUCTION

Dans le cadre d'études sur la décomposition de matière organique dans le sol, on a déterminé la cinétique de production de CO_2 aux dépens de substrats radioactifs [1] et on a établi la répartition de la radioactivité dans les diverses fractions organiques obtenues par transformation de ces substrats [2 3 4]. Faisant suite à ce programme de recherches, il a paru intéressant de suivre la décomposition dans le sol des pigments foliaires radioactifs. Cette étude, dont les résultats sont exposés ici a permis d'établir que ces pigments ne se décomposent que lentement et que la transformation des chlorophylles est différente de celle des pigments caroténoïdes.

II. MÉTHODES EXPÉRIMENTALES

Pour obtenir les pigments radioactifs on sépare du ray-grass radioactif la fraction des substances solubles [1] et on l'extrait de façon exhaustive à l'éther sulfurique fraîchement distillé. On réunit les extraits éthérés, on les lave plusieurs fois à l'eau distillée, on sèche au Na_2SO_4 anhydre et on évapore à sec. On reprend le résidu d'évaporation par 2 ml d'acétone et on sépare les pigments par chromatographie sur papier Whatman 3 MM, utilisant comme solvant un mélange d'éther de pétrole (p.eb. = 30–50°) et de pro-

* Travail effectué sous les auspices de l'Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (I.R.S.I.A.).

panol dans le rapport volumétrique 100/1 [5]. On laisse migrer le solvant pendant une heure environ. Le β-carotène a dans ces conditions un $R_f = 1{,}0$ tandis que le R_f des chlorophylles *a* et *b* sont respectivement 0,70 et 0,46. On achève la purification de la chlorophylle *b* en découpant la papier suivant une ligne médiane entre les chlorophylles et on plonge le papier contenant la chlorophylle *b*, par son bord découpé, dans un mélange d'éther de pétrole (p.eb. = = 30–60°)/chloroforme dans les proportions volumétriques 3/1. On sépare ainsi à l'état pur la chlorophylle *b*. On obtient le β-carotène libre de toutes impuretés en éluant à l'acétone le pigment jaune qui migre avec le front du premier solvant.

Pour la décomposition dans le sol des deux pigments ainsi obtenus, on opère selon la méthode décrite précédemment, de même que pour la mesure de dégagement de $C^{14}O_2$, pour le fractionnement de la matière organique du sol, pour le dosage du carbone, et pour la détermination de la radioactivité. La quantité introduite de substrat correspond à 4 mg C [1 2 3 4].

III. RÉSULTATS ET DISCUSSION

Ainsi que cela a été fait pour d'autres substrats radioactifs, il s'indiquait d'abord de déterminer la cinétique de formation de $C^{14}O_2$ aux dépens des pigments foliaires. Le tableau 1 donne les quantités de $C^{14}O_2$ formées dans le sol par décomposition de la chlorophylle *b* et du β-carotène. On y remarque que, même après 30 jours, la quantité de $C^{14}O_2$ produit ne s'élève qu'à 9,51% pour la chlorophylle *b* et à

TABLEAU 1

Dégagement de $C^{14}O_2$ par décomposition dans le sol de la chlorophylle *b* et du β-carotène				
Jours	Chlorophylle *b* val. in. 6.470		β-Carotène val. in. 2.180	
	c/m *	% **	c/m *	% **
2	250,3	3,87	54,5	2,50
7	220,6	3,41	80,6	3,70
14	50,1	0,80	42,9	1,97
30	92,5	1,43	64,7	2,97
total	613,2	9,51	242,3	11,14

* La radioactivité est exprimée en cent coups par minute (c/m).

** Le pourcentage de radioactivité est exprimée par rapport à la radioactivité initiale du substrat.

11,14% pour le β-carotène. Ces valeurs sont très faibles comparées à celles que l'on obtient à partir du glucose [2 4] dont environ 80% se retrouve à l'état de CO_2 après la même période. Ceci indique que les pigments foliaires ne sont que lentement décomposés dans le sol.

Ainsi que cela avait été établi pour les substances solubles, les protéines ainsi que les hémicelluloses [4], on a déterminé la teneur en carbone et le taux de radioactivité des cinq fractions humiques du sol, obtenues aux dépens des pigments foliaires.

Le tableau 2 donne la répartition de la radioactivité de ces fractions, à la fin de l'expérience en 30 jours.

TABLEAU 2

Radioactivité des fractions humiques du sol obtenues aux dépens des pigments		
Substrat	Chlorophylle *b* C^{14}	β-Carotène C^{14}
Radioactivité * initiale	6.470	2.180
résiduelle **	1.098 (16,97%)	509,9 (23,38%)
subst. solubles ***	4.372,8 (67,58%)	851,2 (59,90%)
Fractions humiques †	Répartition de la radioactivité ††	
F	7,80	16,36
HS	4,51	28,87
HR	22,42	15,42
RS	5,89	12,59
RR	59,38	26,80

* Radioactivité exprimée en cent coups par minute.

** Pourcentage, par rapport à la radioactivité initiale, de l'ensemble des fractions humiques.

*** Pourcentage, par rapport à la radioactivité initiale, de l'ensemble des substances solubles dans l'alcool à 80 et 20%.

† Signification des abréviations: F = acides fulviques;
HS = fraction hydrolysable des acides humiques;
HR = fraction non hydrolysable des acides humiques;
RS = fraction hydrolysable de l'humine;
RR = fraction non hydrolysable de l'humine.

†† Répartition de la radioactivité exprimée en pourcentage, par rapport à la radioactivité résiduelle du sol.

Pour ce qui regarde la radioactivité des fractions humiques (tableau 2) on observe une grande différence d'un pigment à l'autre. On note que le pourcentage de radioactivité des acides fulviques provenant du β-carotène est double de celle des acides fulviques formés aux dépens de la chlororphylle *b*.

Lorsqu'on compare les résultats obtenus par décomposition de la

chlorophylle *b* à ceux relatifs aux fractions équivalentes produites par décomposition du glucose [4], on constate que la répartition de la radioactivité dans HR, la fraction non hydrolysable des acides humiques, représente 22,42% de la radioactivité totale, tandis que la même fraction provenant du glucose après 30 jours de décomposition, ne représente que 4,75%. L'inverse se présente pour HS, l'humine hydrolysable, qui pour la chlorophylle *b* est de 4,51% de la radioactivité totale, tandis que pour le glucose cette fraction représente 29,8%.

Comparée au glucose la décomposition du β-carotène présente le même pourcentage de radioactivité dans HS, cependant que HR est beaucoup plus élevé avec 15,42%. Ceci indique que HR se forme plus facilement aux dépens des pigments que du glucose. En ce qui concerne la fraction RS, l'humine hydrolysable, on constate pour les deux pigments une répartition de loin inférieure à celle du glucose; cependant la fraction RR, l'humine non hydrolysable, est très importante dans le cas de la chlorophylle *b* où elle atteint 59,38%, contre 13,8% pour le glucose. La fraction RR dans le cas du β-carotène occupe une valeur intermédiaire avec 26,80%.

RÉSUMÉ

La décomposition dans le sol de pigments foliaires C^{14}, chlorophylle *b* et β-carotène est très lente comparée à la décomposition du glucose, de l'hemicellulose et de la cellulose.

Cette étude a, en outre, permis d'établir que la formation des substances non hydrolysables HR et RR est plus aisée aux dépens des pigments que de glucose, c'est l'inverse pour la formation des substances hydrolysables.

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ÉTUDE DE LA DÉCOMPOSITION DE LA MATIÈRE ORGANIQUE DANS LE SOL AU MOYEN DE CARBONE RADIOACTIF *

V. DÉCOMPOSITION DE CELLULOSE ET DE LIGNINE

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I. INTRODUCTION

Dans le cadre d'études sur la décomposition de matière organique dans le sol, on a établi la répartition de la radioactivité dans les fractions humiques obtenues par transformation du glucose, des pigments foliaires, des substances solubles, des protéines végétales et des hemicelluloses [7 10 8 11]. Faisant suite à ce programme de recherches on a abordé l'étude de la décomposition dans le sol de la fraction cellulosique radioactive, qui, chez les monocotylées est composée d'environ 80% de cellulose et de xylanes et de 20% de lignine. Cette étude, dont les résultats sont exposés ici, permet d'établir que la décomposition de la lignine est profondément différente de celle de la cellulose, tant en ce qui regarde la cinétique de décomposition qu'en ce qui concerne la nature des substances humiques produites.

II. MÉTHODES EXPÉRIMENTALES

1. *Production des substrats radioactifs*

A. Fraction cellulosique ($C^{14}O_2$). On opère comme décrit précédemment [9] mais au lieu de ray-grass on prend une plante adulte de riz, dont la partie aérienne pèse environ 5 g et on porte la durée de culture dans le liquide nutritif à 4 jours. On utilise 5 mc $BaC^{14}O_3$.

* Travail effectué sous les auspices de l'Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (I.R.S.I.A.).

B. Fraction cellulosique ((PhaC^{14}). On prépare une fraction cellulosique dont la lignine représente 38% de la radioactivité en suivant la technique de Brown et Neish [3]. Des feuilles de riz de 1 g de poids humide dont on a coupé les racines sont immergées par leurs extrémités dans 200 μl (50 μc) d'une solution de phénylalanine (PhaC^{14}). Après 3 heures on réintroduit une même quantité d'eau, puis 3 heures plus tard on ajoute 5 ml d'une solution nutritive d'Hoagland et Arnon et on laisse la plante 4 jours sous cloche au voisinage de 8 lampes au néon de 20W.

Sur une partie aliquote de la fraction cellulosique radioactive préalablement lavée à l'eau, à l'alcool et à l'éther, on détermine la radioactivité de la lignine selon le procédé de Klason. La répartition de radioactivité entre la cellulose et la lignine pour les fractions cellulosiques synthétisées à partir de $C^{14}O_2$ et de PhaC^{14} est résumée au tableau 1.

TABLEAU 1

Répartition de la radioactivité dans la fraction cellulosique selon le procédé de synthèse utilisé			
Fraction	Radio-activité *	Cellulose (81% carbone)	Lignine (19% carbone)
		% radio-activité	% radio-activité
Fraction cell. ($C^{14}O_2$) †	1.516	90,28	9,72
Fraction cell. (PhaC^{14}) ††	10,3	61,80	38,20

* Radioactivité exprimée en μc.

† Fraction cellulosique radioactive synthétisée en laissant la plante quatre jours en atmosphère de $C^{14}O_2$ (5 mc).

†† Fraction cellulosique radioactive synthétisée après quatre jours par absorption par la plante d'une solution de PhaC^{14} (50 μc).

Avant d'être introduites dans le sol, les fractions cellulosiques C^{14} ont été broyées au mortier avec un peu de sable fin.

C. Lignine C^{14}. On obtient celle-ci à partir de la fraction cellulosique ($C^{14}O_2$) suivant le procédé de Klason par digestion à température ordinaire de 2 heures dans H_2SO_4 72% suivi d'une hydrolyse pendant 12 heures à 100° C dans H_2SO_4 *N*. Le résidu, brun jaune, obtenu après centrifugation, constitue la lignine.

D. Cellulose C^{14}. On prépare la cellulose C^{14} à partir de la fraction cellulosique ($C^{14}O_2$) en oxydant la lignine par le nitrobenzène en solution alcaline. Selon la technique de Freudenberg modifiée par Brown, Tanner et Stone [4], on opère sur 50 à 100 mg de la fraction cellulosique que l'on introduit dans une bombe à hydrogénation de 15 ml de capacité. On ajoute 300 μl de nitrobenzène et 5 ml de NaOH 2*N*. Après chauffage avec agitation pendant 3 heures à 160° C on refroidit la bombe et on centrifuge la suspension. La solution servira à la séparation des aldéhydes aromatiques C^{14}, tandis que la précipité blanc, lavé plusieurs fois à l'eau, l'alcool et l'éther donne finalement une poudre blanche de cellulose C^{14}. Cette cellulose C^{14}, comme dans le cas de la lignine C^{14} de Klason, est sans doute partiellement dénaturée par ce traitement.

E. Vanilline C^{14}, syringaldéhyde C^{14}, *p*-hydroxybenzaldéhyde C^{14}. Ces trois aldéhydes aromatiques sont des constituants essentiels de la lignine. Tandis que la lignine des gymnospermes ne donne que la vanilline, celle des angiospermes donne un mélange de vanilline et de syringaldéhyde qui représente 25 à 45% du poids de lignine dosé selon Klason. Les balles de maïs donnent un mélange de 4,5% de vanilline, de 2,6% d'aldéhyde syringique et de 1,4% de *p*-hydroxybenzaldéhyde [1].

On sépare ces trois aldéhydes l'une de l'autre de la façon suivante: On porte la solution alcaline de nitrobenzène dont il est question en D à 50 ml. On l'extrait plusieurs fois à l'éther pour en éliminer le nitrobenzène et on porte la solution à pH = 7,0; on extrait 4 à 5 fois avec 20 ml d'éther sulfurique et on réunit les extraits éthérés qu'on évapore jusqu'à faible volume. On reprend avec un peu d'alcool et on étend la solution sur une ligne droite à 5 cm en bordure d'une feuille de Whatman no. 4. On chromatographie, utilisant comme solvant la phase supérieure du mélange butanol/acétone/tampon NH_3, $(NH_4)_2CO_3$ 1,5 *N* (4/3/3 v/v) [12]. Après 24 heures on sèche le chromatogramme et on le met sous film RX Gevaert Structuric DX. Après 3 jours on développe le film, l'emplacement des aldéhydes sur le papier correspondant aux zones sensibilisées sur le film (Fig. 1). On découpe le chromatogramme en bandes, suivant ces zones, et on élue séparément chacune de ces bandes avec une solution alcoolique à 50%.

On porte à pH = 2,0 la solution primitive de pH = 7,0 par addition de quelques gouttes d'HCl 6*N*. On sature la solution de NaCl et on extrait 3 fois à l'acétate d'éthyle. On chromatographie comme il vient d'être décrit et on sépare ainsi 10 substances (ou groupes de substances) (Fig. 2), dont certaines

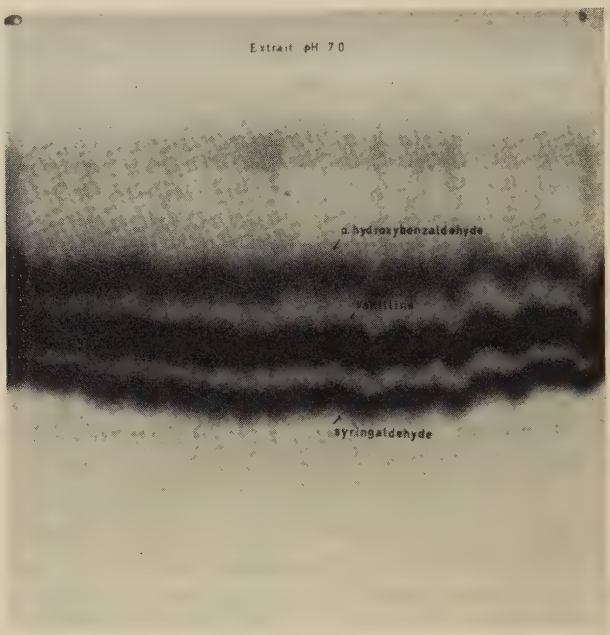

Figure 1.

sont identifiées. On groupe au tableau 2 quelques propriétés des substances mises en évidence, ainsi que la répartition de leur radioactivité. Les substances qui sont connues sont celles de la zone 5 (mélange d'acide vanillique, d'acide 3-4-5-trimethoxybenzoïque), celle de la zone 6 (acide *p*-hydroxybenzoïque) et celle de la zone 7 (acide férulique).

2. *Decomposition des substrats organiques dans le sol et mesure de la radioactivité des fractions humiques*

On mélange chacune des substances, produites par voie biologique, à une quantité de sol de prairie équivalente à 4 g de poids sec [9] et on les soumet à décomposition pendant 30 jours à 25° C. La quantité introduite de substrat correspond à 4 mg C. Pour l'extraction, le fractionnement et le dosage des substances on opère comme précédemment [2]. On sépare ainsi les acides fulviques (fraction F), les acides humiques hydrolysables (fraction HS) et non hydrolysables (fraction HR), l'humine hydrolysable (fraction RS) et non hydrolysable (fraction RR). On mesure avec un Flow-counter "Nuclear" (1 coup = 3,4 dés.) la radioactivité de chacune de ces fractions sur

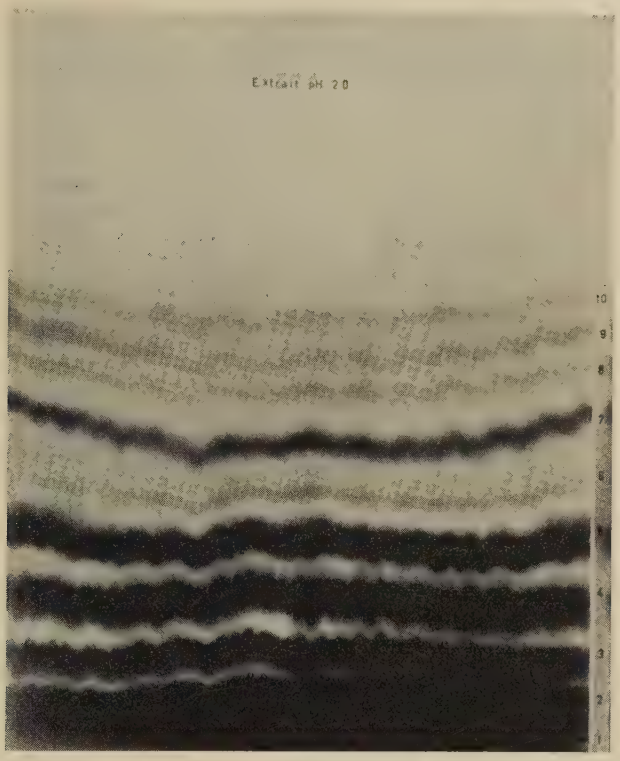

Figure 2.

le $BaCO_3$ obtenu après combustion suivant Van Slyke - Folch [8 13] de chacune de ces fractions.

RÉSULTATS ET DISCUSSION

Le tableau 3 donne les résultats de la décomposition de la fraction cellulosique radioactive synthétisée respectivement aux dépens de $BaC^{14}O_3$ et de phénylalanine C^{14}, dont on se rappelera que la pourcentage de radioactivité dans la lignine est respectivement 9,72 et 38,5 (tableau 1). Bien que dans les deux cas on ait introduit dans le sol le même poids de substrat radioactif, on constate cependant que la fraction cellulosique ($C^{14}O_2$) ne laisse que 28% de son activité dans le sol, tandis que la fraction cellulosique (PhaC^{14}) en maintient 44,5%.

TABLEAU 2

Chromatographie des extraits * neutre et acide obtenus après dégradation alcaline de la fraction cellulosique ($C^{14}O_2$) **				
Extrait à pH = 7,0	R_f	% radio-activité	Révélateur 2.4-D.N.P.	Substances
	0.58	4,13	orange	syringaldéhyde
	0.64	5,23	orange	vanilline
	0.72	3,67	orange	*p*-hydroxybenzaldéhyde
Extrait à pH = 2,0	R_f	% radio-activité	Révélateur *p*-nitraniline	
***				
1	0	17,92		
2	0.08	27,14		
3	0.18	10,85		
4	0.26	11,18		
5	0.34/ 0.38	9,89	bleu/ pourpre	ac. vanillique/ac. 3.4.5-trimethoxy-benzoïque
6	0.45	4,22	rouge	ac. *p*-hydroxybenzoïque
7	0.52	5,70	bleu	ac. ferulique
8				syringaldéhyde
9		traces		vanilline
10				*p*-hydroxybenzaldéhyde

* Se référer au texte pour la préparation des extraits et l'isolement des substances par chromatographie.

** Radioactivité totale des extraits: $44{,}5.10^6$ c/m.

*** Les chiffres correspondent à ceux de la figure 2.

Il faut admettre que dans la fraction cellulosique la cellulose et la lignine se décomposent avec des cinétiques différentes. Cela ressort encore des deux dernières colonnes du tableau 3 où l'on voit que 70% de la radioactivité de la lignine C^{14}, après 30 jours, se retrouvent sous forme de fractions humiques, tandis que pour la cellulose C^{14} il en reste 20%.

La répartition de la radioactivité dans les différentes fractions du sol montre encore qu'après décomposition de la fraction cellulosique ($C^{14}O_2$), les trois quarts de la radioactivité se répartissent de façon à peu près équivalente entre les acides fulviques F, les fractions hydrolysables des acides humiques HS et de l'humine RS. Il est intéressant de souligner que les fraction humiques du sol après dégradation de la cellulose C^{14} présentent une répartition de radioactivité analogue. Il n'en est pas de même pour la fraction cellulosique (PhaC^{14}) et pour la lignine C^{14}. Dans ces deux cas les frac-

TABLEAU 3

Radioactivité des fractions humiques du sol obtenues aux dépens de la fraction cellulosique et de ses constituants, lignine et cellulose radioactives

Substrat	Fraction cellulosique C^{14}		Lignine C^{14}	Cellulose C^{14}
	$C^{14}O_2$ *	PhaC^{14} **		
Radioactivité *** initiale	2.370	411	5.600	1.110
résiduelle †	660 (28%)	183 (44,5%)	3.939 (70,3%)	221 (20%)
Fractions humiques ††	Répartition de la radioactivité §			
F	22,59	12,41	10,33	25,30
HS	26,09	16,75	12,96	24,66
HR	9,49	23,29	35,72	5,0
RS	25,93	14,83	5,60	35,09
RR	15,88	32,70	35,37	10,14

* Fraction cellulosique radioactive synthétisée à partir de $BaC^{14}O_3$.

** Fraction cellulosique radioactive synthétisée à partir de PhaC^{14}.

*** Radioactivité exprimée en mille coups par minute.

† Pourcentage de l'ensemble des fractions humiques par rapport à la radioactivité initiale, abstraction faite des substances solubles dans l'alcool à 80 et 20%.

†† Signification des abréviations: F = acides fulviques; HS = fraction hydrolysable des acides humiques; HR = fraction non hydrolysable des acides humiques; RS = fraction hydrolysable de l'humine; RR = fraction non hydrolysable de l'humine.

§ La répartition de la radioactivité est exprimée en pourcentage par rapport à la radioactivité résiduelle du sol.

tions F, HS et RS ont une radioactivité moindre tandis que la radioactivité des fractions HR et RR est sensiblement plus élevée. Ces deux fractions non hydrolysables HR et RR totalisent 56% de l'activité résiduelle dans la décomposition de la fraction cellulosique (PhaC^{14}) tandis qu'elles totalisent 71% dans la décomposition de la lignine C^{14}.

Le tableau 4 donne les résultats de la décomposition dans le sol des substances de dégradation de la lignine C^{14}, c'est-à-dire la syringaldéhyde C^{14}, la vanilline C^{14} et la *p*-hydroxybenzaldéhyde C^{14} et l'ensemble des substances extraites à pH = 2,0 citées au tableau 2. On observe immédiatement que ces substrats ne sont pas entièrement décomposés en trente jours comme le témoigne la radioactivité de la fraction soluble, qui représente encore 12,3 à 25,9% de celle du substrat original. La répartition de la radioactivité dans le sol montre que pour ces quatre substrats les fractions F, HR et RR sont prédominantes. En fait, à la différence près que la fraction F est ici plus importante, cette répartition est

TABLEAU 4

Radioactivité des fractions humiques du sol, obtenus aux dépens des produits de dégradation de la lignine C^{14}, de l'acide benzoïque C^{14} et du glucose C^{14}

Substrat *	Syringaldéhyde C^{14}	Vanilline C^{14}	*p*-Hydroxy-benzaldéhyde C^{14}	Extrait à pH = 2,0	Acide benzoïque C^{14}	Glucose C^{14}
Radioactivité ** initiale	887	2.211	988	5.796	6.470	6.100
résiduelle ***	151 (17 %)	389 (17,6%)	156 (15,8%)	903 (15,6%)	951 (14,7%)	690 (11,3%)
subst. solubles †	118,5 (13,3%)	273,4 (12,3%)	258,7 (25,9%)	884 (15,2%)		
Fractions humiques ††	Répartition de la radioactivité §					
F	25,15	31,36	28,13	23,88	19,98	20,28
HS	12,99	10,44	11,52	20,15	24,85	29,80
HR	28,33	29,66	26,57	21,19	9,65	4,75
RS	7,15	9,72	13,82	12,51	28,61	31,26
RR	26,37	18,80	19,95	22,25	16,89	13,80

* Voir dans les méthodes expérimentales la synthèse des aldéhydes aromatiques radioactives.

** Radioactivité exprimée en mille coups par minute.

*** Pourcentage par rapport à la radioactivité initiale de l'ensemble des fractions humiques.

† Pourcentage par rapport à la radioactivité initiale des substances solubles dans l'alcool à 80 et 20%.

†† Signification des abréviations: voir tableau 3.

§ La répartition de la radioactivité est exprimée en pourcentage par rapport à la radioactivité résiduelle du sol.

analogue à celle obtenue aux dépens de la lignine C^{14}. On observe, par contre, que pour le glucose C^{14} (v. Bibl.[8]) et l'acide benzoïque C^{14} ce sont au contraire les fractions HS et RS qui ont la radioactivité la plus élevée.

Le tableau 5 donne de façon plus détaillée la répartition de la radioactivité entre la fraction hydrolysable et la fraction non hydrolysable des acides humiques obtenues aux dépens des substrats étudiés. On y constate que pour le glucose C^{14} et la cellulose C^{14},

TABLEAU 5

Rendement et répartition de la radioactivité dans les fractions hydrolysable (HS) et non hydrolysable (HR) des acides humiques.

Substrat	HS	HR	Rendement *
Fraction cellulosique $C^{14}(C^{14}O_2)$	82,23	17,77	8,8
Fraction cellulosique $C^{14}(PhaC^{14})$	41,83	58,17	17,8
Lignine C^{14}	32,79	67,21	34,2
Cellulose C^{14}	83,14	16,86	5,9
Acide benzoïque C^{14}	72,02	27,98	5,0
Glucose C^{14}	86,24	13,76	3,9
Subst. isolées de l'extrait à pH = 7,0			
syringaldéhyde C^{14}	33,06	66,94	7,0
vanilline C^{14}	26,04	73,96	7,0
p-hydroxybenzaldéhyde C^{14}	30,25	69,75	6,0
Extrait à pH = 2,0	48,75	51,25	6,4

* Le rendement en acides humiques est donné par rapport à la radioactivité du substrat initialement introduit.

82 à 86% de la radioactivité se retrouve dans la fraction HS. On se rappelera à ce propos que le même cas se présente pour les substances solubles C^{14} du ray-grass, les hémicelluloses C^{14} et les protéines C^{14} [8]. L'acide benzoïque C^{14} donne une répartition du même type, avec, toutefois, une plus faible activité dans HS (72%). Par ailleurs, on observe pour la lignine C^{14} et pour les aldéhydes aromatiques C^{14} que 66% à 73% de la radioactivité se concentre dans HR.

Le tableau 5 donne encore le rendement des acides humiques C^{14} formés aux dépens de la fraction cellulosique et de ses constituants. On constate que la quantité d'acides humiques formés aux dépens de la lignine C^{14} représente 34,2% de la radioactivité primitive,

tandis que pour la cellulose, elle n'atteint que 5,9%. Ceci explique les différences de rendement que l'on obtient à partir des deux différentes fractions cellulosiques, dont les pourcentages de radioactivité de la lignine sont respectivement 9,72% et 38,2%. Dans ce dernier cas le rendement atteint 17,8% et dans le premier cas seulement 8,8%.

Il semblerait que certaines chaînes polyphénoliques interviennent telles quelles dans la formation des substances qui composent la fraction non hydrolysable des acides humiques HR. C'est ainsi que les dérivés phénoliques simples: syringaldéhyde, vanilline et la *p*-hydroxybenzaldéhyde donnent une fraction HR qui représente 4% à 5% de la radioactivité initiale, tandis que pour la lignine cette fraction correspond à 25,1%.

Les résultats obtenus soulèvent encore le problème de l'origine des acides humiques. Lorsqu'on étudie par chromatographie les fractions HS et HR on constate à la suite de Bremner [2] que HS est constituée dans sa majeure partie par des acides aminés, tandis que la fraction HR est composée d'acides et d'aldéhydes phénoliques obtenus aussi par oxydation de la lignine [6 12]. Ceci reviendrait à dire que les protéines microbiennes formées aux dépens des hydrates de carbone seraient à l'origine des substances quaternaires de la fraction HS, tandis que la fraction non hydrolysable HR serait essentiellement formée aux dépens de la lignine ou des substances phénoliques qui la constituent: vanilline, syringaldéhyde ou *p*-hydroxybenzaldéhyde. Bien que l'on en soit réduit à des hypothèses quant au mécanisme même de la formation des substances de la fraction HR on peut admettre, avec Flaig [5], une condensation des di-phénols, due à l'action des phénoloxydases d'origine microbienne.

RÉSUMÉ

On a étudié au moyen de carbone radioactif la décomposition dans le sol de la fraction cellulosique du riz, la décomposition de la cellulose ainsi que celle de la lignine et de ses constituants: la vanilline, la syringaldéhyde et la *p*-hydroxybenzaldéhyde. Il en ressort que dans la fraction cellulosique, la cellulose et la lignine se décomposent avec des cinétiques différentes: dans un même laps de temps se dégradent 80% de cellulose pour 30% de lignine. En outre, 5,9% et 34,2% de la radioactivité se retrouvent dans les acides humiques respectivement pour la cellulose et pour la lignine. La répartition de la radioactivité dans la fraction hydrolysable HS et dans la fraction non hydrolysable HR des acides humiques est encore différente pour la cellulose et pour la lignine. On constate en effet que 66 à 73% de cette radioactivité se retrouvent dans la fraction HR, après décomposition de la lignine, tandis qu'on observe dans le cas de la cellulose, que 82% de la radioactivité se concentrent dans la fraction HS. On observe la même répartition de radioactivité pour la lignine par la décomposition de la vanilline, de la syringaldéhyde et de la *p*-hydroxybenzaldéhyde. Sachant que la fraction hydrolysable HS est constituée dans sa majeure partie d'acides aminés, tandis que la fraction non hydrolysable HR est formée d'acides et d'aldéhydes phénoliques composant la lignine, les résultats obtenus suggèrent que les substances quaternaires retrouvées dans la fraction HS proviennent des protéines microbiennes formées aux dépens des hydrates de carbone, tandis que la fraction non hydrolysable HR serait principalement formée aux dépens de lignine ou de ses substances phénoliques.

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